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# Thermophilic and Thermoduric Microorganisms, with Special Reference to Species Isolated from Milk

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# THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS, WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK

## I. REVIEW OF LITERATURE

A. H. ROBERTSON

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TECHNICAL BULLETIN No. 130

# THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS, WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK

## I. REVIEW OF LITERATURE[1]

A. H. ROBERTSON[2]


### ABSTRACT

**Spore-forming thermophiles are widely distributed in nature. Physiologically and morphologically many of them resemble the common aerobic spore-formers and some investigators suggest that the former are derived from the latter by gradual acclimatization. Thermophilic actinomyces are common in nature also.**

**Only one non-spore-forming thermophile, *Lactobacillus thermophilus* Ayers and Johnson (1924b), has been described. Non-spore-forming species with unusual thermal resistance have been found among the streptococci, the sarcinae, and the lactic acid bacteria.**


### INTRODUCTION

Certain species of micro-organisms among the algae and bacteria, and less commonly among the molds and yeasts, have been found which are resistant to temperatures that are abnormally high for the existence of living protoplasm. Not only do the organisms survive, but in many cases they grow normally at and above 60° C. In this respect these forms differ from the normal species in these groups. Thermophilic bacteria normally belong to spore-forming types, tho non-spore-forming types have been described.

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[1]A portion of the thesis submitted to the Graduate School at Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy. The entire contribution appears in four parts, two of which, Technical Bulletins Nos. 130 and 131, are published by the New York State Agricultural Experiment Station, and two, Bulletins Nos. 274 and 275, by the Vermont Agricultural Experiment Station. The author acknowledges the many helpful suggestions offered during the course of the present investigation by Dr. R. S. Breed of the New York Station and by Dr. H. B. Ellenberger and Dr. B. F. Lutman of the Vermont Station.

[2]Formerly Assistant in Research (Bacteriology) at this Station; now Research Dairy Bacteriologist at the Vermont Agricultural Experiment Station, Burlington, Vt.

Sonnerat (1774)[3] first reported the existence of life at unusually high temperatures. According to an early statement, he saw living fish at 69° C in a thermal lake on the Island of Luzon, Philippine Islands. Wyman (1867) claimed that Sonnerat's observation was incorrect as determined by later visits to this lake.

A strictly thermophilic bacterium was first isolated from the Seine River by Miquel (1879). Miquel (1881, 1882, 1888) also was first to isolate thermophilic bacteria from soil and feces. The bacterial flora of canned goods was first investigated by Prescott and Underwood (1897, 1898) who probably failed to isolate any strict thermophiles.

The present work has been stimulated largely by Dotterrer's (1923) recognition of the presence of an unusually heat-resistant or thermophilic (heat-loving) flora in pasteurized milk. Soon after the World War, numerous laboratories engaged in milk control work began reporting the sporadic appearance of large numbers of small, punctiform, white colonies on agar plates. These so-called "pin-point" colonies were associated with pasteurized milk.

Fay (1927) has pointed out that the term "pin-point" colony may superficially characterize a number of different types of organisms as they grow on the agar plates. The term is quite unsatisfactory as it does not distinguish between the true thermophiles, the thermotolerant flora, and certain mesophiles, because several of these species under certain conditions may form punctiform colonies. The writer has observed spore-forming thermophiles whose colonies also might be considered "pin-point."

Usually public health officials, after observing the presence of this type of colony growth, advised the milk companies that some faulty operation had occurred at the plant which was responsible for the high bacterial count. When reports of similar conditions in several other cities in this country were given proper consideration, it was recognized that the cause of the "pin-point" colonies was not the result of so simple a thing as the failure properly to wash and sterilize the pasteurizing equipment. In some plants a series of samples were secured at different intervals during the pasteurization process which showed that the bacterial count of the milk increased during pasteurization.

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[3]Refers to Bibliography, Page 21.

The purpose of this investigation is to study the non-spore-forming, thermophilic and thermoduric species associated with milk. The results will appear in four parts as follows: I, a review of the literature on the thermophilic and related micro-organisms; II, a discussion of the heat resistance of micro-organisms; III, a description of the characters of the isolated organisms, some of which may frequently cause the "pin-point" colonies on the pasteurized milk plates; and IV, a report showing the effect of the age of the culture on the heat resistance of non-spore-forming bacteria.

Portions of the literature have been reviewed by Ambroz (1910), Noack (1912), Morrison and Tanner (1922, 1924), Krohn (1923), Tanner (1924b), Magoon (1926a), and Tanner and Harding (1926b). Therefore, under each classification by source only a short statement of the literature will be presented. The contributions will be reviewed according to the habitats from which the micro-organisms were isolated, as follows: (1) Milk, with special reference to bacteria; (2) waters, including fresh, thermal, and mineral; (3) feces, sewage, and heating manure piles; (4) soil; (5) canned goods; and (6) miscellaneous sources. The nature of certain contributions does not permit their being reviewed according to habitat. Therefore, they are considered in a separate section and because of their general scientific importance follow the preceding review of the literature on isolation according to habitat.

In order that there may be no misunderstanding of the terms applied to thermophilic and unusually heat-resistant bacteria, it is best to adhere to some classification. Usually macroscopic observation is the only means employed to detect optimum growth conditions. The classification is as follows: Strict thermophiles, optimum growth above 55°C and failure to grow below 37°C; facultative thermophiles, optimum growth between 45° and 55°C; thermotolerant species, optimum growth between 37° and 45°C; and thermoduric mesophiles, optimum growth between 20° and 37°C, but will survive in relatively large numbers in raw milk (at least 90 per cent survival) at temperatures as high as 62.8°C for 30 minutes.

The writer realizes that the term thermoduric mesophile will include some members of the genus *Lactobacillus*, some strains of *Escherichia coli* (Escherich) Castellani and Chalmers, and some so-called varieties of *Streptococcus lactis* (Lister) Löhnis, as well as many of the aerobic and anaerobic spore-forming group. High thermal resistance alone is not a character of sufficient importance to justify

establishing a new genus or species. Therefore, certain strains of some species are described merely as possessing thermoduric properties. A variety name or symbol is preferred unless there are two or more characters that distinguish the organisms in question from related forms.

## THERMOPHILIC AND THERMODURIC MICROORGANISMS IN MILK

### I. SPORE-FORMING SPECIES

Since the present work deals primarily with heat-resistant bacteria in milk, the literature will be discussed from this viewpoint, altho it is recognized that the original habitat of spore-forming thermophilic species is not the milk itself. The poorly washed milk utensils with the partially dried-on casein and albumin may be suitable places for the development of thermophiles. Also the dust from the air is a probable source of thermophiles.

That thermoduric spore-forming bacteria are common types in pasteurized, sterilized,[4] and boiled milk is well established by the studies of Hüppe (1884), Löffler (1887), Gruber (1888a, 1888b), Adametz (1890), Hesse (1890), Strub (1890), Petri and Maassen (1891, 1898), Pictet and Weyl (1891), Frankel (1893), Flügge (1894), Wróblewski (1895), Weber (1900), Dunbar and Dreyer (1900), Ferguson (1902), Rogers (1905), Knüsel (1908), Ford and Pryor (1914), Lawrence and Ford (1916), Laubach, Rice, and Ford (1916), Lisk (1924), and Robertson, Yale, and Breed (1926). The species found most frequently are members of the hay- and potato-bacillus groups.

Flügge (1894) was probably working with some of the thermophilic species, altho he did not recognize them as such. Leichmann (1894) was among the first to recognize that he had isolated a bacterium with thermophilic properties from milk. After heating the milk from 12 to 15 hours at 50°C, it coagulated, but the coagulum was different from that produced by typical strains of *Streptococcus lactis*. He isolated from this mass a slime-producing, facultative-thermophilic spore-former which grew best between 40° and 50°C. Rabinowitsch (1895) first isolated strict thermophiles from milk. Among the other workers who have isolated spore-forming thermophiles from milk and its products are Gorini (1895), Oprescu (1898), Weber (1900), Sames (1900), Schardinger (1903), Ambroz (1910),

[4]Term used in the practical sense.

Eckelmann (1917), Bergey (1919), Morrison and Tanner (1922), Tanner and H. G. Harding (1926a, 1926b), Clarke and Dougherty (1926), Eckford (1927) and H. G. Harding (1927). Because of the common characteristics possessed by all thermophilic spore-formers, a general description of the species in this group appears later.

## II. NON-SPORE-FORMING SPECIES

That certain of the pathogenic staphylococci are more resistant to heat than other non-spore-forming pathogens has been established by the early work of Sternberg (1887), Van Geuns (1889), and De Man (1893), and more recently confirmed by Rosenau (1908), Patzschke (1916), and Park (1927). Hauser (1887) was perhaps the first to recognize the unusual heat resistance of some species of the micrococci. Adametz (1890) was among the first to observe sarcinae in sterilized milk. Dyar (1895) isolated a yellow micrococcus (probably a sarcina) from sterilized milk. Stubenrath (1897) studied sarcinae from other sources than milk and noticed their heat resisting properties. Hashimoto (1901) reported the isolation of micrococci from sterilized milk. Russell and Hastings (1902b) undoubtedly were studying a sarcina when they described a micrococcus, the thermal death point of which is 76°C in milk. Schweitzer (1903) observed the heat resistance of sarcinae in milk. Ayers and Johnson (1910, 1913) unquestionably had sarcinae among their acid-forming and alkali-forming groups which survived pasteurization. More recently Fenger, Cram, and Rudnick (1920) have shown that micrococci (probably sarcinae) occasionally survive in the heat treatment used in preparing surgical ligatures and sutures. In a preliminary report, the writer (1923) isolated some micrococci among which were sarcinae from pasteurized milk plates.

Marshall (1897) was among the first to observe the customary lactic acid fermentation in pasteurized milk similar to that occurring in raw milk. A sufficient number of the lactic acid bacteria survive the pasteurization process to produce normal lactic acid fermentation. Weiss (1899), Hashimoto (1901), Freudenreich and Thöni (1904), Rogers (1905), Adeloff (1907), Wolff (1908), Ayers and Johnson (1910, 1913), Shippen (1914), Hammer (1914), Gorini (1915, 1921), Peiser (1915), Brown and Peiser (1916), Weigmann, Wolff, Trensch and Steffen (1916), Patzschke (1916), Orla-Jensen (1919, (1921), Robertson (1923), Hammer and Baker (1923, 1926), and Fay (1927) have noted the thermal resistance of some of the lactic acid

species in milk. Ayers, Johnson, and Davis (1918) discovered that eight of their cultures of pathogenic streptococci survived 140° F for 30 minutes. A further study of these eight cultures showed that they could be distinguished from the other pathogenic species by certain cultural characters. Van Tieghem (1881), Hashimoto (1901), Lingelsheim (1912), Holman (1914), Bleyer (1923), and Johnson and Exworthy (1925) have isolated various streptococci, with unusual heat resistance, from milk or other substances. Beckwith and Eddie (1924) observed that hemolytic streptococci of human origin are not always killed by the pasteurization temperatures commonly used. Salter (1921) and Sears and Benson (1923) have reported similar findings. Several species of the genus *Lactobacillus* grow best at temperatures between 38° and 44°C (Bergey, 1923).

Colon bacilli with high thermal resistance have been observed by De Jong and De Graff (1906), Zelenski (1906), and Gage and Stoughton (1906). Ayers and Johnson (1915) studied the thermal death points of 174 cultures of *Escherichia coli* from one or more of the following sources: Human and cow feces, flies, cheese, milk, and cream.

Twiss (1920) reported that some of the colon and paratyphoid group survived pasteurization. Immediately Krumweide and Noble (1921) refuted this contention, suggesting that some error had been introduced into Twiss' work.

Greig-Smith (1909) and Downs (1925) have observed *Micrococcus albus* (Rosenbach) Buchanan frequently in condensed milk. Orla-Jensen (1919) reported the presence of sarcinae in sweetened condensed milk. Savage and Hunwicke (1923) have isolated micrococci most frequently from both sweetened and unsweetened condensed milk. Hunwicke and Jephcott (1925) have submitted data on the numbers and types of bacteria killed in the roller process of milk drying. Brannon and Prucha (1927) found two cultures which survived pasteurization among the 46 non-spore-forming organisms isolated. One of these was similar to Russell and Hastings' (1902b) organism. Among 21 non-spore-forming species which had been named before they were received at the laboratory, *Sarcina lutea* was found to survive the pasteurization temperature in milk.

Altho Jacobsen (1918), H. A. Harding (1923), Yates (1923), and Yates and Glover (1923) were probably dealing with a thermophilic flora in milk which they described as producing "pin-point" colonies on agar plates, Dotterrer (1923) first recognized the possibility of the development of a heat-loving flora in the pasteurizer with the in-

creased time of operation. Dotterrer took samples of milk from the holder at five-minute intervals when the same holder was used for five successive fillings. The plate count of the milk held at 143°F for 30 minutes indicated the normal reduction in the first filling only. In the other four fillings the count did not change much, altho in one case it was greater at the end than at the beginning of the holding period.

The work on this new "pin-point" flora of pasteurized milk has been reviewed by Tanner and H. G. Harding (1926) from the contributions by Ayers and Johnson (1923), Robertson (1923), Van Horn (1924), Taylor (1924a, 1924b), Hungerford and H. A. Harding (1924), Tanner (1924a), Adams and H. A. Harding (1924), H. A. Harding and Ward (1924), Ayers and Johnson (1924a, 1924b), and Swenarton (1925, 1926). The description of a new species, *Lactobacillus thermophilus* (Ayers and Johnson, 1924b), which grew best at pasteurizing temperatures explained one of the causes of "pin-point" colonies. The presence of "pin-point" colonies has been detected in the milk from individual producers and has been found both in certified and in medium and poor quality milk. More recently Clarke and Dougherty (1926) have confirmed some of the preceding observations on non-spore-forming thermophiles. Fay (1927) has described three types of thermo-tolerant saccharophilic organisms which form characteristic "pin-point" colonies.

## THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS FROM RIVERS, CANALS, AND HOT SPRINGS

### I. BACTERIA

The earliest reference to a strictly thermophilic bacterium dates back to Miquel (1879) who attributed the property of thermal resistance to a different form of protoplasm. Prior to this Cohn (1875) isolated a facultative thermophile, *Beggiatoa nivea* Rabenhorst, from the sulfur springs at Aargau, Germany. Among the others who have isolated spore-forming thermophiles from similar sources are Certes and Garrigou (1886), Winogradsky (1887), Claassen (1888), Moniez (1893), Teich (1895), Pretti (1896), Kedzoir (1896), Miyoshi (1897a, 1897b), Davis (1897), Harshbarger (1897), Oprescu (1898), Michaelis (1899), Setchell (1903), Kehler (1904), Catterina (1904), Benignetti (1905), Skotschko (reviewed by Benignetti), Brazzola (1906), Andres (1906), Falcioni (1907), Tirelli (1907), De Kruijff (1909, 1910), Georgevitch (1910a, 1910b, 1911), Ambroz (1910, 1913), Nègre

(1913a, 1913b), Rössler (1918), Dufrenoy (1921), Morrison (1921), Morrison and Tanner (1922), and Elion (1924). Cantacuzène (1910) has demonstrated the presence of a thermophilic spirochete in the springs at Dax.

### II. ALGAE

Hooker (1813) was among the first to note the presence of *Oscillariae* growing at 98°C in one of the thermal springs in Iceland. Fluorens (1846) also identified algae which came from an equally high temperature. Algae and related forms of plant life are frequently found growing naturally at temperatures ranging from 60° to 75°C in various thermal waters. Ehrenberg (1858), in addition to algae, has described motile rotifers, some members of the *Polygalaceae*, and living *Eunotia Sancti Antonii* at 63° to 65°C. Long (1822) reported the presence of numerous insects at the sides and bottoms of pools at 55.6° to 65.6°C. A small bivalve testaceous animal adhered to the plants and lived at these temperatures also. Among others who have observed thermophilic algae are James (1823), Agardt (1827), Corda (1835-36), Schwabe (1837), Meneghini (1843), Gervais (1849), Kützing (1849), Humboldt (1852), Stachey (1854), Hooker, J. D. (1854), Lauder-Lindsay (1861), Cohn (1862), Brewer (1866), Wyman (1867), Bickmore (1868), Hayden (1870-83), Lyman (1874, 1878), Parry (1874), Wood (1874), Dyer (1874), Moseley (1874), Hoppe-Seyler (1875), Archer (1875), Jones (1875), Skey (1877), Dana (1880), Serres (1880), Semper (1881), Junghühn (——), Griffiths (1882), Rogers (1884), Certes and Garrigou (1886), Perroncito and Varalda (1887), Hansgrig (1887), Nordstedt (1888), Kerner von Marilaun (1888), Weed (1887-88, 1889), Baring-Gould (——), Moseley (1892), Buscalione (1895), Pfeffer (1897-1904), and Lowenstein (1903).

## THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS IN FECES, SEWAGE, AND MANURE

Miquel (1888) isolated the first thermophile from feces. Others who have studied thermophiles from the above sources are Dehérain (1884), Globig (1888a, 1888b), Burrill (1889), Schlösing (1892), Rabinowitsch (1895), Macfadyen and Blaxall (1894a, 1894b, 1899), Baruchello (1897a, 1897b), Müller (1898), Herzberg (1899) Dupont (1902a, 1902b), Tsiklinski (1903), Bruini (1905), Skotschko (——), Anitschkow (1906), Bardou (1906), Ambroz (1910), Eckelmann (1917), Bergey (1919), Veillon (1922), Damon and Feirer (1925), and Greer (1926). Greer found that many of the common anaerobes could

grow rapidly at 50°C and a few could grow a little less abundantly at 55°C.

Miquel regarded the thermophiles in feces as unimportant, since their greatest activity occurred at temperatures above that of the human body. Anitschkow did not find the thermophiles common in the feces, but their presence could be easily demonstrated by enrichment methods.

## THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS IN THE SOIL

Miquel (1888) has priority in isolating thermophiles from soil also. Others who have found thermophiles in the soil are Globig (1888a, 1888b), Macfadyen and Blaxall (1894a, 1894b, 1899), Rabinowitsch (1895), Christen (1895), Cambier (1896), Baruchello (1897a, 1897b), Schillinger (1898), Oprescu (1898), Tsiklinski (1899a, 1899b), Sames (1900), Gilbert (1904), Benignetti (1905), Skotschko (——), Blau (1906) De Kruijff (1909, 1910), Ambroz (1910, 1913), Pringsheim (1911), Koch and Hoffmann (1912), Nègre (1913a, 1913b), Kroulik (1913), Eckelmann (1917), Krohn (1923), and Feirer (1927).

De Kruijff (1909), under tropical conditions, obtained atmospheric nitrogen fixation with thermophiles at 55°C. In the tropics, flasks of water reach a sufficiently high temperature during the day for the rapid growth of thermophiles. A maxium temperature of 62°C has been recorded. Spores of thermophiles when inoculated into bouillon grew during the middle of the day. At least nine hours during the day conditions are extremely favorable for decomposition by the spore-forming thermophiles. As the temperature approaches the maximum for the psychrophiles, their activity diminishes and simultaneously the activity of the thermophiles increases. At 11 o'clock in the evening a temperature of 48°C has been recorded for swamp water. Top soil temperatures favor the growth of thermophiles during the greater part of the day.

## THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS ASSOCIATED WITH THE CANNING INDUSTRY

The need for canned goods in this country was first felt during and immediately after the Civil War period, as indicated by the growth of the canning industry. For a long time success in canning was regarded as a mystic art. Later work has shown that the lack of

success in many cases was caused by the presence of thermophilic species which were not killed by the heat penetration. Duckwall (1899) submitted the first reliable treatise on bacteriology as applied to the canning and preserving of food products. Prescott and Underwood (1897, 1898) were perhaps the first to describe a group of species which they had isolated from canned goods. They found two species of micrococci and seven bacilli which were probably merely thermoresistant species. Russell (1895b) pointed out that underprocessing was the cause of a gaseous fermentation in canned goods. That canned goods often contained thermophiles was recognized by Ott (1904) and confirmed by Cathcart (1906).

A period of 14 years elapsed with few contributions, particularly in this country. Stimulated by the canning experiences during the World War, a deluge of new material, a large proportion of which treats of the causes and the methods of controlling spoilage, has been published. Only the important contributions will be mentioned.

Members of the hay- and potato-bacillus groups have been found most frequently in canned goods. New species, some of which are true thermophiles, have been described by Barlow (1912), Weinzirl (1919), Hunter and Thom (1919), Cheyney (1919), Esty and Williams (1919), Normington (1919), Donk (1919, 1920), Bunyea (1921), Wyant (1923), Wyant and Tweed (1923), Bitting (1924), Weinzirl (1924), Cameron and Esty (1925), and Fellers (1926). Molds, yeasts, actinomyces, sarcinae, streptococci, and micrococci have been isolated also.

## THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS FROM MISCELLANEOUS SOURCES

### I. SILAGE

Burrill (1889), Albert (1893), and Griffiths (1894) have shown that the spore-forming thermophiles are associated with silage fermentation. Temperatures between 56°C and 70°C favor the formation of sweet silage. Ambroz (1910) found thermophiles in silage, but no data are submitted.

### II. HAY

Cohn (1893a), Miehe (1906, 1907a), Dügelli (1906), Schütze (1908), Molisch (1908), and Miehe (1909) have isolated thermophiles from heating green hay. Boekhout and Ott de Vries (1908, 1909a) found that the heating was not wholly dependent on the thermophilic

species but instead was caused by an oxidation process catalyzed by the presence of iron.

### III. COTTON

Zopf (1892), Haepke (1893), and Cohn (1893) have investigated the thermophiles in cotton. Zopf isolated a thermo-tolerant rod and Cohn found a heat-resistant micrococcus.

### IV. TOBACCO

A number of investigators, among whom are Cohn (1893), Haepke (1893), Dávalos (1892), Behrens, (1894, 1896 1901), Koning (1897, 1898), Vernhout (1898, 1899), Loew (1899, 1900a, 1900b, 1901), Delacroix (1907), and Jensen (1908), have associated thermophiles with tobacco fermentations. Boekhout and Ott de Vries (1909b) clarified the uncertainty of tobacco heating by showing that it depended on an oxidation process activated by the presence of iron.

### V. AIR, DUST, AND FILTH

Globig (1888b), Macfadyen and Blaxall (1894a, 1894b, 1899), Rabinowitsch (1895), Sames (1900), Bardou (1906), De Kruijff (1909), and Bergey (1919) have isolated thermophiles from dust and air.

### VI. BREAD

The sliminess of improperly baked bread has been investigated by Laurent (1885), Kretschner and Niemslowicz (1889), Uffelmann (1890), Russell (1898), Vogel (1897), Eckles (1899, 1902), Juckernach (1900), Thomann (1900), König and Spickermann (1902), Budinoff (1903), Fuhrmann (1906), Ambroz (1910), and Kaiser and Delaval (1911). During the baking of bread, the maximum temperature is usually not over 105°C. Probably certain substances in the bread aid in retarding the passage of water into the cell. *Bacillus panis* (Vogel) Migula has been isolated most frequently. Other species which have been isolated commonly are members of the hay- and potato-bacillus groups.

Cohn (1893) and Rabinowitsch (1895) found thermophiles in grains. The heating of meal has been investigated by Zopf (1892), Holliger (1902), Levy (1904), Maurizio (1906), and Teichert (1907). Schardinger (1903, 1907) isolated them from cereal and from stuffed foods, such as sausages and frankfurts. Laxa (1898, 1900), Poŭpě (1898), and Kosscowicz (1912, 1913) have found them in sugar solutions. Ambroz (1910) associated a decomposition of hyacinth bulbs with a thermophilic growth. Sames (1900) described a thermophile associ-

ated with certain discharges from the human body. Greig-Smith (1921) isolated a thermophile from fermenting tan-bark. Cohn (1893) associated thermophiles with the heating of hops.

## PAPERS OF GENERAL SCIENTIFIC INTEREST

Göbel (1923) obtained a higher survival of *Bacillus pyocyaneus* Gessard cells in a bouillon suspension than in a physiological salt suspension when they were subjected to the same temperatures. The lower the dilution of the organisms, the greater was the percentage of the organisms surviving. In other words, the resistance of the individual cell in a dilute suspension was greater than in a concentrated suspension.

Bleyer (1923) heated cultures of streptococci at 50°, 56°, 60°, 65°, 70°, and 80°C for one hour in a water bath. After inoculating the heated cultures into the blood stream of white rats, he recovered streptococci in a number of cases from the liver, spleen, and heart blood.

Behrens (1923) maintained that there was an optimum concentration of cells above or below which a greater percentage of killing occurred when the culture was subjected to high temperatures. Oerskov (1925, 1926) found that the older cultures, i. e., 24 hours old versus freshly inoculated (not more than 2 to 3 hours old), were more resistant to heat.

Eijkmann (1908) was first to show the "S" shape of the rate-of-killing curve when bacteria were subjected to temperatures approaching their thermal death point. Just (1877) observed the germination of seeds after exposure to high temperatures after heating in dry air and in liquids. Porodko (1927a, 1927b) has presented additional data on the rate of killing of seeds by heat and their ability to germinate afterwards. Boycott (1919) obtained S-shaped curves for the rate of killing of multicellular organisms (tadpoles) when subjected to heat.

Tanner and Wallace (1925) obtained the growth curves of three species of thermophilic bacteria at 20°, 37°, and 55°C in plain broth. At 20°C the growth was irregular and not as rapid as at 37° or 55°C. No definite peak in the curve was obtained. At 37°C the period of maximum growth was reached in from 16 to 30 days. At 55°C growth was very rapid, many of the cells dying soon after the period of active growth had passed. Cells of thermophilic species growing at 55°C wear themselves out quickly, the cultures often becoming sterile.

Preheating the medium to 55°C before inoculation always shortened the lag phase of growth. At 55°C the lag phase was from three to four hours long when inoculated with old cultures but was almost eliminated when inoculated with rapidly growing cultures.

Koser (1927) has demonstrated a bacteriophage which is active against a thermophilic species. Golikowa (1926) reported that the thermophilic species can adapt themselves to unusual temperature conditions. The characteristics of the various species isolated are unstable. Group agglutinins for thermophiles could be developed in dog serum.

Most bacteriologists agree that the thermal death points of bacterial species must be expressed in some form of temperature-time relations. That thermal resistance was dependent on the nature of the medium containing the bacteria was perhaps first demonstrated by Von Esmarch (1888) and has been confirmed by later investigators.

Buchanan, Thompson, Orr, and Bruett (1918) submitted a formula for determining the temperature at the center of a can of food during the process of sterilization. The formula may be applied at any time except during the first few minutes. Bruett (1919) found that blanching was not an effective method of killing the spores in canned goods. Burke (1919) first called attention to the delayed germination of spores after they had been heated. Bigelow and Esty (1920a, 1920b) first pointed out that the initial number of spores influenced the time required for sterilization. They confirmed the work of Buchanan *et al* (1918) on the influence of the free hydrogen-ion concentration on the heat resistance of the spores.

Considerable variation has been observed in the thermal resistance of different strains of the same species as well as different concentrations of the same strain by Shanly (1915), Esty and Williams (1919), Dickson, Burke, and Ward (1919), Dickson, Burke, Beck, Johnson, and King (1922), and Esty (1923). Some workers attribute this to a higher resistance of a few cells, since more than 95 per cent of the cells are killed in a relatively short time at an exposure just below their maximum thermal resistance. Burke (1923) explained this property by the probable impermeability of the cell wall to water.

Differences in the rate of heat penetration in the processing of canned foods were first pointed out by Bigelow (1920) and his collaborators, Bohart, Richardson, and Ball. These differences depend

on the nature and consistency of the contents of the cans, variation in the size of the can, the initial temperature, the retort temperature, and the cooling and heating methods. Bigelow and Cathcart (1921) called attention to the wide variations in the pH values for individual canned foods and to the changes in the pH values during processing.

Dickson, Burke, and Ward (1919) found that the addition of 5 per cent lemon juice to the medium did not prevent the formation of toxin by *Clostridium botulinus* Van Ermengen (1896) but did lower the thermal death points. Bigelow (1921) in his studies on the processing of canned goods first used the logarithmic thermal death time curves to express the rate of death of the organisms when heated.

Magoon and Culpepper (1921) summarized some of the factors affecting the penetration of heat into cans as follows: (1) Size of vessel, (2) ability of the container to radiate heat, (3) concentration of the sugar or starch within the container, (4) mobility of the contents of the container, (5) mobility of the heating and cooling medium, and (6) the shape of the container.

Esty and Cathcart (1921), reporting on the influence of hard and soft pyrex glass tubing for heating cultures, found that unbuffered solutions caused considerable change in the pH. Heating mixtures of $Na_2HPO_4$ and $KH_2PO_4$ in hard glass tubes did not affect the hydrogen-ion concentration during the heating, while prolonged heating in soft glass tubes dissolved out alkali in excess of the amount which could be controlled by the buffer salts. Heating in alkali solution of this mixture in soft glass caused a greater lowering of the hydrogen-ion concentration than acid solutions. A neutral solution did not change appreciably during the heating, even in soft glass tubes. Heating juices of canned vegetables in soft glass tubes affected the pH less than in hard glass tubes.

Magoon and Culpepper (1922) pointed out that some of the variations in thermal resistance of micro-organisms in canned foods was dependent on variations in the initial temperature of the substances in the cans and the pressures and vacuums within the cans. Weiss (1921a) found that young moist spores were more resistant than old ones, and that spores one month old were three times as resistant as those five months old. Esty and Meyer (1922) reported that young moist spores, probably those of the first generation, appeared to be the most resistant. Magoon (1926a) found that older spores were more resistant than the young ones. Among some of the fac-

tors influencing the heat resistance of spores were the humidity and the temperature at which the spores were held.

Tanner and Dack (1922) found that the spores of *Clostridium botulinum* survived much higher temperatures when exposed to dry heat than have been reported for its resistance to moist heat, as reported by Weiss (1921b). Tanner and McCrea (1923) observed that a longer time was required to destroy the spores of *Clostridium botulinum* of the same age in open tubes than in tubes exhausted and sealed. After Burke (1923), Esty (1924) recognized the presence of especially heat-resistant individuals in the spore suspensions. Their presence explains the skips which occur when a series of tubes containing the same suspension of spores is subjected to the same heat treatment at a temperature in the proximity of their thermal death point. Hastings, Fred, and Carroll (1926) suggested that the presence of skips and stops could be partially overcome by the use of proper methods. Some modifications were the use of a neutral heating medium, a uniform cell suspension, the absence of contamination in the heated cultures, and perfect conditions for growth after the heat treatment.

Weinzirl (1924) showed that aerobic spore-formers when inoculated into sterile meat and incubated at 37°C never produced typical putrefaction. Anaerobic spore-formers were regarded as responsible for the typical putrefaction of meat. Starin (1926) found that the spores of *Clostridium botulinum* tended to lose not only their viability but their toxigenic powers also when subjected to heat. Viljoen (1926) observed a protective action exerted by sodium chloride in concentrations up to 4 per cent on bacterial spores in heated pea liquor.

## GENERAL DESCRIPTION OF SPORE-FORMING THERMOPHILES

Before summarizing the distribution of the various species according to their habitats, a brief statement of the characters of the spore-forming thermophiles is given. The rods may be long or short, thick or slender, motile or non-motile, Gram positive or negative, mostly non-capsulated, and are arranged singly, paired, or in chains. Thread forms are rather common. Roeser (1890) showed that the typhoid bacilli assume long thread shapes when grown at their maximum temperatures. Aerobes, facultative anaerobes, and strict anaerobes are found. The spores may be either central or terminal and oval

or round. The growth on agar may or may not be luxuriant and may or may not be pigmented. Usually the growth is dull white or brownish. Carbohydrates are usually fermented, but gas may or may not be formed. Gelatin may or may not be liquefied. Nitrates may or may not be reduced to nitrites and ammonia. Milk may or may not be coagulated and peptonized. Starch is usually hydrolyzed. Usually the species are not pathogenic. Indol and hydrogen sulfide are sometimes formed. Frequently growth does not occur on the agar and potato slants.

The above description might just as well be applied to the common spore-forming species. Dupont (1902) has shown that *Bacillus subtilis* (Ehrenberg) Cohn and *B. mesentericus ruber* Globig grow at 50°C. Blau (1905) found that the spores of *B. subtilis* germinate at 60°C after a long time. After 48 hours at 55°C the spores of *B. subtilis* formed thin grayish-yellow colonies. *B. parvus* Neide (1904) also grew under similar conditions in 24 hours. *B. megaterium* De Bary (1884) and *B. subtilus* grew at 50°C. After 48 hours at 50°C, *B. ruminatus* Gottheil (1901) produced incipient signs of growth. Greer (1926) found that many of the anaerobic spore-formers grew at 50°C and a few could grow at 55°C.

Rabinowitsch (1895), Schillinger (1898), and Tsiklinski (1903) regarded the thermophiles as merely varieties of the common non-thermophilic species. These species were simply thermo-tolerant, since they had adapted themselves to new temperature conditions (Schillinger.) Because of their resistance to adverse conditions and their power of adaptibility, they are found widely distributed in nature. Tsiklinski believed that the facultative thermophiles would become strict thermophiles if continually grown at high temperatures. A facultative thermophile would merely be an intermediate stage. Eckelmann (1917) has demonstrated a partial loss and recovery of the ability to resist heat among some of her thermophiles. After growing in liquid media, their heat-resistance decreased, but after growth on solid media for a long time their former heat resistance was regained.

## DISTRIBUTION OF SPECIES DESCRIBED BY PREVIOUS WORKERS

Spore-forming thermophilic rods can be associated with all the sources which have been considered, i.e., milk, water, feces, soil, canned goods, silage, and various fermenting organic materials. Mi-

crococci are more frequently associated with milk and its products. Some workers have found them in canned goods. Less commonly they have been found in unsterile surgical sutures and ligatures, fermenting cotton, and in water in which beans have been boiled.

Thermophilic actinomyces have been found by Globig, Karlinski, Rabinowitsch, Kedzoir, Tsiklinski, Sames, Bruini, Gilbert, Miehe, Schütze, Nègre, Velich, Bergey, Lieske, Krohn, and Eckford. Isolations have been made from one or more of the following places: Soil, fresh water, sulfur springs, sewage, manure, fresh feces, milk, canned goods, hay, air, and the Sahara sands.

Thermophilic species are not uncommon in mineral springs. Cohn, Certes and Garrigou, Winogradsky, Karlinski, Teich, Falcioni, Miyoshi, and Dufrenoy have isolated thermophiles from sulfur springs. Hoppe-Seyler, Miyoshi, and Dufrenoy have found them in water containing iron.

Bardou (1906) and Ambroz (1913) have described denitrifying species. Elion (1924) described a sulfate-reducing thermophile. Pringsheim (1913), Kroulik (1913), and Viljoen, Fred, and Peterson (1926) have found thermophiles associated with the destruction of cellulose. Thermophiles in soil, manure, and fermenting green fodders are quite likely to be cellulose fermenters.

Anaerobic thermophiles have been isolated by Oprescu (1898), Tsiklinski (1903), Nègre (1913a), and Veillon (1922). More recently Greer (1926) showed that many of the common anaerobes could grow rapidly at 50°C. Nègre concluded that all obligate thermophilic bacteria were obligate aerobes and that facultative thermophiles were facultative aerobes, a statement with which other investigators do not agree.

## DISTRIBUTION OF LIFE WITH REFERENCE TO TEMPERATURES

MacDougal (1921) and MacDougal and Working (1921) have reported a most interesting observation on the high temperature record for endurance and growth in *Opuntia*. In the second paper a temperature of 56.5°C in the tissue and 58.0°C for the air was reported. Bates and Roeser (1924) showed that variation in the resistance of tree seedlings to excessive heat was dependent on the lack of moisture in the soil to maintain turgor and the seedlings tipped over at the ground line. Certain trees, and naturally also their seedings, are better adapted thru their root systems to over-

come the lack of moisture in the surface soil of semi-arid, hot regions. Sachs (1864) observed that plants were killed at an air temperature of 51°C in 10 to 30 minutes, while 45° to 46°C in water kills them in 10 minutes.

The distribution of life according to temperatures has been well summarized by Davenport and Castle (1895). The *Protista* have the highest maximum temperature of any group, it being above 60°C for active organisms in water. The *Ciliata* show the decidedly low temperature of 42°C. Among the *Metazoa*, the highest maxima recorded are 44° and 45°C for some insects (Plateau, 1872). For the great majority of the *Metazoa* the maximum temperature lies between 30° and 40°C. The green algae are less resistant than the blue-green algae, the maximum temperature for the former being about 70°C and for the latter about 80°C. Probably some of the blue-green forms can survive the boiling temperature of water.

## SUMMARY

The literature on thermophilic and thermoduric micro-organisms has been reviewed. Spore-forming thermophiles are widely distributed in nature. They have been isolated from the following habitats: Thermal springs, fresh waters, canal waters, sewage, fresh animal and human feces, infant feces (one to five days old), cultivated and uncultivated soil (particularly garden soil), market milk, canned goods, heating manure piles, fermenting malt, meal, bran, hay, cotton, tobacco, silage, tanbark, hops, grains, and the "fullmasse" of sugar solutions. Ordinarily they are not common, but their presence can easily be demonstrated by enrichment methods.

Physiologically and morphologically they resemble the common aerobic spore-formers. Some investigators suggest that the property of unusual thermal resistance has been acquired by the aerobic spore-forming species and that after the acquisition of this new character they are recognized as thermophiles. Natural conditions probably induce certain of the species with the greatest powers of adaptability to modify their existence and gradually to take on new properties such as growth at higher temperatures. Thermophilic actinomyces have also been isolated from a variety of sources.

Only one non-spore-forming thermophile, *Lactobacillus thermophilus* Ayers and Johnson (1924b), has been isolated. Milk is the only recognized source of this species at present. Non-spore-forming species with unusual heat resistance have been found among the

streptococci, the sarcinae, and the lactic acid bacteria. The unusual thermal resistance of a few strains of *Escherichia coli* has been noted.

The highest temperatures at which life exists has been observed among the algae and has been recorded as 98°C. Thermophilic bacteria grow best between 55° and 75°C. The maxium temperatures for the *Metazoa* are 44° to 45°C.

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# THERMOPHILIC AND THERMODURIC MICROORGANISMS, WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK

## II. THE THERMAL RESISTANCE OF MICROORGANISMS

A. H. ROBERTSON

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**The ability of living protoplasm to survive high temperatures depends, in part at least, on its low moisture content. Data are presented and literature cited which suggest that: (1) changes in the nature of the cell-wall membrane, (2) changes in the cell produced by growth in concentrated sucrose solutions and (3) changes evolved through acclimatization processes may be instrumental in effecting a cell with a low moisture content and consequently a higher thermal resistance.**

# BULLETIN 274: THERMOPHILIC AND THERMODURIC MICROORGANISMS, WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK[1]

## II. THE THERMAL RESISTANCE OF MICROORGANISMS

A. H. Robertson

A broad speculative field lies before the investigator who attempts to explain the heat resistance of microorganisms. The life of the cell is so exceedingly intricate, so inter-dependent on the composition of the medium and so adaptable as a result of necessity, even under what most bacteriologists call normal conditions of growth, that when abnormally high temperatures are used the study becomes all the more complicated. The application of heat in general stimulates the activity of all substances. The reactions between the cell and the surrounding medium become more violent. The increased metabolic activity of the cell simultaneously tends to decrease its longevity (Tanner and Wallace, 1925).

Bacteriologists generally recognize that the thermal resistance of the cell is in part, though perhaps not wholly, dependent on its moisture content. In order to develop the thought more clearly the early literature supporting these claims will be reviewed. Then it will be shown that (1) the nature of the cell wall, (2) the osmotic pressure of nutrient solutions and (3) the adaptability of species may be among the factors which aid in establishing a lower moisture content and, consequently, a higher thermal resistance.

During the course of this investigation the influence of the possible changes in the equilibria between the different combinations of the proteins within the cell wall and the various acid and alkali radicals on the heat coagulation of proteins has not been considered. The large protein molecules and their ability to combine and absorb tend to diminish the deleterious effects of the presence of small quantities of acid and alkali. Further studies are planned to consider this phase of the problem with respect to living protoplasm. For references to and summaries of contributions in this field with special consideration of

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milk proteins, one may seek papers by Leighton and Mudge (1923), Leighton and Deysher (1923) and Sommer (1923).

## HEAT RESISTANCE OF SPOREFORMING MESOPHILES

### Heating Dry and Moist Spores

The causes of thermal resistance have usually been studied among the larger sporeforming microorganisms because changes in the protoplasm within the cell can be detected more easily. Doyère (1842) found that various animalcules (rotifers and tardigrades) which are ordinarily killed in water at 50° C. may, after long drying, be heated in air to 120° C. without a 100 percent death rate. Pasteur (1861) observed that the spores of some molds when dry survive 120° C. Damp uredospores are killed at 58.5° to 60° C. but dry ones withstand 128° C. (Hoffmann, 1863). Schützenberger (1879) discovered that dry yeast might be heated to 100° C. without being killed but that moist yeast was killed at 60° C. Dallinger (1880) reported that the dry spores of various flagellata withstood a temperature from 10° to 25° C. higher than that which the same spores could resist in liquid media.

Zopf (1885) observed the unusual resistance of bacterial spores at the boiling point of water and suggested that this property was related to the spore contents. The composition of the vegetative cell must differ from that of the spore. Arloing, Cornevin and Thomas (1882), Flügge (1886) and Gruber (1888a, 1888b) found that the spores were more easily killed in aqueous solutions than in air. The references cited thus far indicate that the vitality of the spores when heated depends on the moisture content.

### Heat-Absorbing Protective Coatings About Cells

Von Esmarsch (1889) suggested that the spores surround themselves with a firm protective coating which partially prevents the penetration of heat. Lewith (1890) regarded the heat capacity of any protective covering as insignificant because it is impossible to conceive of a coating of sufficient thickness or of proper composition (formed by bacteria in ordinary nutrient broth) protecting as small an object as a living bacterial cell against the penetration of heat. The same reply might be made to Wolff's (1908) and Gorini's (1915, 1921) statements that the lactic acid bacteria in milk surround themselves with a protective covering when heated. While protective coatings are more

likely to be formed in milk than in non-saccharin broth cultures, such protective coatings probably protect the albumin from coagulation when heat is applied either by withdrawing the moisture already within the cell or by retarding the passage of moisture inward through the cell wall.

### HEAT-COAGULABLE ALBUMIN WITHIN THE CELL

Hellmich (Lewith, 1890) isolated a heat-coagulable albumin-like protein from the cell contents. Nencki and Drymont's (1886) anthrax protein contained a heat-coagulable portion. Since it was evident that the cell contained a heat-coagulable protein, Lewith undertook to show under what conditions this substance would not coagulate when subjected to heat. Hass (1876) obtained by drying in vacuum an egg albumin which was not coagulable at 155° C., partly coagulable at 160° C. and completely coagulated at 170° C. Lewith determined experimentally that the temperature at which the coagulation of pure albumin occurs is inversely proportional to its water content. The following table is taken from Lewith's paper:

| Egg albumin | Coagulation temperature |
|---|---|
| In aqueous solution | 56° C. |
| In 25 percent water | 74° to 80° C. |
| In 18 percent water | 80° to 90° C. |
| In 6 percent water | 145° C. |
| Without water | 160° to 170° C. |

Theoretically it would appear that any non-toxic substance possessing a mild dehydrating influence on the cell would tend to form an albumin of lower moisture content.

## INFLUENCE OF CELL (SPORE) WALL ON HEAT RESISTANCE

Cramer (1891) and Benecke (1912) reported that the moisture content of the spores was lower than that of the vegetative cells. In the cell during spore formation, a dehydration of the albumin portion of the protoplasm apparently occurs. The free water becomes either combined or absorbed and the protoplasmic portion becomes more concentrated.

Cramer (1894) maintained that the dense protoplasm is surrounded by a mantel of cellulose and other starch-like substances which have a great affinity for moisture. The mantel is alcohol-soluble and saturated with fat-like substances. The resistance of spores to dry heat depends on the retention of the hygroscopic moisture by the cell mantel while the resistance to moist heat depends on the retarding

effect which the spore wall exerts on the penetrating moisture as it goes through to the albumin.

## THE RELATIVE PERMEABILITY OF THE VEGETATIVE CELL AND SPORE WALLS

That internal changes in the protoplasm do occur during spore formation have been established by the early observations of De Bary (1884), Lewith (1890), Bütschli (1884, 1889, 1890), Brauer (1894) and Davenport and Castle (1895). These changes consist essentially in the formation of a granular, optically denser, compact mass of protoplasm which stains irregularly. Finally the outline of the spore wall appears and then a special staining technic is required to stain the spore.

Cramer's theory is substantiated by the relative rates of dye penetration through the wall of the vegetative cell and through that of the spore.

The acid-fastness of the tubercle bacillus is related to certain properties of the cell wall which make it more difficult to stain. The common stains penetrate less easily but when stained properly the cells are not easily decolorized. The tubercle organism is considered also one of the most heat-resistant of the non-sporeforming pathogens. The property of acid-fastness and of heat resistance appear to be dependent on the cell wall and again suggest the probability that the cell wall of this organism retards the penetration of water. The inability of the stain to penetrate may be dependent on the fat-like composition of the cell wall.

The dead spores also are more easily stained than the living ones (Ross, 1909; Proca, 1909; Kayser, 1912; Seiffert, 1922; Fulmer and Buchanan, 1923; Burke, 1923a, and Koser and Mills, 1925), indicating that the spore wall has lost its power to retard the passage of aqueous solutions. The unusual heat resistance of a few individual spores which survive the majority thermal death point undoubtedly depends on the greater impermeability of the cell wall to water (Burke, 1923b).

The spore is not incomparable to a seed. The moisture content of the seed is always relatively low as compared to that of the plant before the seed begins to form. At the end of the growing season, the foods are stored in the seed partly as carbohydrates (starch) and partly as fatty substances. Cramer (1894) noted the fat-like material in the mantel of the spore. Seeds likewise can withstand higher temperatures than the vegetative plants (Just, 1877, and Porodko, 1927a, 1927b).

## Desiccation and Resistance

Lewith (1890) has observed that as the moisture content of albumin decreases its coagulation temperature increases. When dehydration of the cell exceeds a certain limit death ultimately ensues. Because desiccation is related to the nature of the spore wall and is one of the factors which enter into sterilization with dry heat, it will be considered at this point.

Liesenberg and Zopf (1892) found that *Streptococcus mesenteriodes* was more easily killed by desiccation when non-capsulated than when capsulated. When grown in saccharin media, the resistance is greater than when grown on a medium containing no sugar. Revis (1910, 1911) found that two types of coliform organisms with mucilaginous type of growth survived the longest period in soil. Revis (1913) asserted that the slime acted as a water-absorbing and water-retaining substance and in this manner promotes the longevity of the species. Löhnis (1913) found that not only spores but bacteria also with slimy walls endure the effects of desiccation very well. Orla-Jensen (1909) observed the protective action of capsules and slime against desiccation.

Giltner and Langworthy (1915-16) concluded that the survival of non-sporeforming bacteria in the air-dry soil is dependent, in part, on the retention by the soil of moisture in the hygroscopic form. They found that if the bacteria are suspended in a solution extracted from rich clay loam before being subjected to desiccation in sand, they live longer than if subjected to desiccation after suspension in a physiological salt solution. The extract from the clay loam contains substances which have a protective action for bacteria against desiccation.

Ficker (1908a, 1908b) and Chester (1907) showed that the medium on or in which the species is last cultivated and the substances on which the desiccation occurs influence its longevity. Briscoe (1912) reported that the differences in longevity were dependent on the amount of hygroscopic moisture retained by the substances on which the desiccation occurs.

Apparently a number of factors among which is capsule formation influence the longevity of the cell when subjected to desiccation. Buchanan (1909) showed that the gum produced by *Bacillus radicicola* in saccharose solutions is closely related to the dextrans that have been described as produced by other groups of bacteria. These complex carbohydrates, usually being colloidal in nature, have an affinity for

water. A capsule of this nature probably retards the passage of water inward as well as outward through the cell wall.

In order to observe the longevity of the culture which had been selected for further study of their heat resistance, a series of cultures[1] on agar slants were held at room temperatures. Each tube was carefully wrapped in paper to prevent evaporation. At different times as indicated in table 1, two of the tubes were selected to determine the ability of the cultures to survive. The growth being relatively scant in some cases, a tube of sterile milk was poured onto the agar slants and these were incubated for a week at 30° C. The yellow and pink sarcinae apparently possess greater resistance against desiccation than the cultures of *Microbacterium lacticum, Streptococcus thermophilus* and *Micrococcus conglomeratus.*

TABLE 1—LONGEVITY OF CULTURES ON NUTRIENT AGAR SLANTS. SLANTS INOCULATED DECEMBER 17, 1924

| ate ted | Species name | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Microbacterium lacticum* | | *Streptococcus thermophilus* | | *Sarcina lutea* | | *Micrococcus conglomeratus* | | *Sarcina rosea* | |
| o. 20, 1925 ..... | + | + | + | + | + | + | + | + | + | + |
| ay 20, 1925 .... | + | + | + | + | + | + | + | + | + | + |
| t. 10, 1925 ..... | + | + | + | + | + | + | — | + | + | + |
| c. 17, 1925 ..... | + | — | + | + | + | + | + | + | + | + |
| o. 25, 1926 ..... | + | — | + | — | + | + | — | — | + | + |
| ay 19, 1926 .... | — | — | + | — | + | + | — | — | + | + |
| y 2, 1926 ..... | — | — | — | — | + | + | — | — | + | + |
| c. 28, 1926 ..... | — | — | — | — | + | + | — | — | + | — |

Sarcinae also survive higher temperatures than the micrococci and the common non-sporeforming rods (except *Microbacterium lacticum* Orla-Jensen, 1919, and *Lactobacillus thermophilus* Ayers and Johnson, 1924b) as indicated by the observation on the species described. The slimy substance which holds the cells together in packets apparently influence the exchange of water between the cell and the medium.

## EFFECT OF OSMOTIC PRESSURE ON HEAT RESISTANCE

### Hypotonic Solutions

Exosmosis is an important factor when microorganisms are subjected to high temperatures, as revealed in tables 2 and 3. The cells used in these experiments were washed from the agar slant with sterile

[1] Later studies revealed that the cultures chosen were members of the species *Microbacterium lacticum* Orla-Jensen (1919), *Streptococcus thermophilus* Orla-Jensen (1919), *Sarcina lutea,* Schröter (1872), *Micrococcus conglomeratus* Migula

distilled water. These were washed and centrifuged twice. One c. c. of this suspension was mixed with 30 c. c. of the sterile substances whose protective actions were to be determined. Five c. c. portions of these suspensions were introduced into five sterile, cotton-stoppered test tubes and subjected to the same temperatures. After heating, the suspensions were plated with nutrient agar containing one percent sucrose. The plates were incubated for two days at 37° C. The number of colonies developing on the agar plates after incubation appear in the accompanying tables.

More cells are killed when heated to the same temperature in distilled water than when heated in a physiological salt solution and more cells are killed in the latter than when heated in a nutrient broth solution containing 16.7 percent sucrose. The influence of the sucrose will be considered under hypertonic solutions.

Five c. c. portions of a non-saccharin nutrient broth culture of *Streptococcus thermophilus* were introduced into 35 c. c. of both sterile distilled water and sterile physiological salt solution. Five c. c. portions of these respective suspensions were heated at 65°, 70°, 75° and 80° C. and a similar protective action was manifested by the physiological salt solution.

TABLE 2—INFLUENCE OF HEATING A SUSPENSION OF *Micrococcus aureus* CELLS IN DIFFERENT LIQUIDS ON THE NUMBERS SURVIVING

| Medium | Number of cells per c. c. surviving* when held for 30 minutes at: 65° C. | 70° C. | 75° C. | 80° C. |
|---|---|---|---|---|
| Distilled water | 3,700 | 21 | 10 | 0 |
| | 2,900 | 36 | 3 | 0 |
| | 7,000 | 64 | 0 | 0 |
| | 5,100 | 27 | 0 | 0 |
| | 6,200 | 119 | 7 | 0 |
| Physiological salt solution | 31,400 | 172 | 3 | 0 |
| | 20,700 | 347 | 0 | 4 |
| | 17,700 | 161 | 22 | 0 |
| | 13,800 | 184 | 1 | 0 |
| | 26,700 | 297 | 0 | 2 |
| Nutrient broth containing 16.7 percent sucrose | 91,000 | 267 | 2 | 3 |
| | 86,000 | 341 | 10 | 7 |
| | 15,000 | 1,710 | 7 | 5 |
| | 171,000 | 410 | 27 | 0 |
| | 103,000 | 373 | 4 | 0 |

* Number is indicated by colony count on agar plates. The initial concentration of the cells was the same for all suspensions.

TABLE 3—INFLUENCE OF HEATING A SUSPENSION OF *Streptococcus thermophilus* IN DIFFERENT LIQUIDS ON THE NUMBERS SURVIVING

| Medium | Number of cells per c. c. surviving* when held for 30 minutes at: 65° C. | 70° C. | 75° C. | 80° C. |
|---|---|---|---|---|
| Distilled water | 150 | 0 | 0 | 0 |
| | 200 | 0 | 0 | 0 |
| | 200 | 0 | 0 | 0 |
| | 216 | 0 | 0 | 0 |
| Physiological salt solution | 1,600 | 40 | 0 | 0 |
| | 1,400 | 77 | 0 | 0 |
| | 900 | 110 | 0 | 0 |
| | 1,720 | 137 | 0 | 0 |

* Number is indicated by colony count on agar plates. The initial concentration of the cells was the same for all suspensions.

## HYPERTONIC SOLUTIONS AND MEDIA OF LOW MOISTURE CONTENT

That various organisms grow in hypertonic solutions (usually either sodium chloride or sucrose) has been observed by Eschenhagen (1889), Reinhardt (1892), Klebs (1896), Wehmer (1897), Gundermann (1898), Karcz (1898), Laxa (1898, 1900), Claassen (1898, 1902), Stadler (1899), Lewandowsky (1904), Kohn (1909), Guillemard (1909), Weichel (1910), Karaffa-Korbut (1912), Orla-Jensen (1919), Brown (1920), Dozier (1924), and Katzu (1925). Weigert (1913), Schlitzer (1905) and Jorns (1907) have grown bacteria on media containing as low as 40 percent moisture. In pickling, canning and preserving, concentrations of salts, sugars and other substances are employed which exert a much greater osmotic pressure than that of a physiological salt solution. Certain species have been isolated under these conditions.

The work by Swellengrebel (1905) suggests that certain species, particularly yeasts, are more sensitive to the germicidal action of various salts than others. A consideration of the data in the papers mentioned above suggests that each species, and even cultures within that species, may respond quite differently to alterations in the osmotic pressure of solutions.

Theoretically, an increase in the concentration of soluble substances in the medium in which the cells are suspended would cause a corresponding increase in the plasmolysis of the cell. Death is caused by plasmolysis alone when the concentration exceeds a certain point which varies both with the dissolved substance and the individual cell. Death may frequently result earlier from other causes.

Plasmolysis of the cell results necessarily from differences in osmotic pressures on each side of the permeable membrane, the cell wall being the membrane. Membranes become less permeable as they get older. The water passes through the cell wall until there is an equalization of pressures. Lewith (1890) has shown that the heat coagulation points of albumin are increased as the moisture content is decreased. If the plasmolyzing substance was non-toxic, its chief effect would be to withdraw the water and produce an albumin with a higher thermal resistance.

Viljoen (1926) has already shown that concentrations of sodium chlorid up to 4 percent in pea liquor have a protective action for bacterial spores against heat. In order to avoid the influence of toxic and dissociating substances, the protective action of sucrose for bacteria against heat has been determined in nutrient broth.

## INFLUENCE OF HYPERTONIC SUCROSE SOLUTIONS OF HEAT RESISTANCE

### PLATING METHOD TO DETERMINE SURVIVAL

The influence of different concentrations of sucrose in ordinary nutrient broth on the number of bacteria surviving when subjected to the same temperatures has been determined. Cultures of *Streptococcus thermophilus, Sarcina lutea, Escherichia coli* (*B. coli*) and *Micrococcus aureus* were grown in nutrient broth containing 23.1 percent cane sugar for 36 hours. The culture was then neutralized with sterile 0.1 normal sodium hydroxid. Five c. c. portions of this broth culture were mixed with 35 c. c. portions of nutrient broths containing the following percentage concentrations of sucrose: 1, 23.1, 33.3, 41.2, and 50. Immediately three c. c. portions of these respective mixtures were placed in a series of sterile, cotton-stoppered test tubes of uniform size and heated at the desired temperatures for 30 minutes. The temperatures used varied because some of the species are more sensitive to heat than

TABLE 4—INFLUENCE OF DIFFERENT CONCENTRATIONS OF SUCROSE IN NUTRIENT BROTH ON THE NUMBER OF CELLS SURVIVING PASTEURIZATION AT DIFFERENT TEMPERATURES

| Culture used* | Numbers of bacteria surviving per c. c. at the respective temperatures in nutrient broth containing the following percentages of sucrose | | | |
|---|---|---|---|---|
| | 1 percent | 23.1 percent | 33.3 percent | 41.2 percent |
| *Streptococcus thermophilus,* culture 23 R, heated at 66° C. for 30 minutes | 116 | 2,000 | 3,500 | 10,000 |
| | 106 | 700 | 2,500 | 10,000 |
| | 326 | 1,000 | 1,000 | 2,000 |
| | 121 | 800 | 600 | 6,000 |
| | 237 | 1,500 | 3,000 | 10,000 |
| | 141 | 600 | 1,500 | 8,000 |
| | 53 | 300 | 2,000 | 15,000 |
| *Escherichia coli,* cuture 1 R, heated at 62° C. for 30 minutes | 20 | 36 | 61 | 600 |
| | 2 | 32 | 47 | 1,200 |
| | 5 | 41 | 150 | 2,000 |
| | 0 | ? | 200 | 2,000 |
| | 1 | 16 | 37 | 2,000 |
| | 3 | ? | ? | 1,000 |
| | 0 | 7 | 70 | ? |
| | 1 | 17 | 45 | 800 |
| | 0 | 5 | 50 | 1,200 |
| *Micrococcus aureus,* culture 6 B, heated at 66° C. for 30 minutes | 10 | 61 | 117 | 70 |
| | 8 | 300 | 162 | 100 |
| | 1 | 110 | 142 | 104 |
| | 31 | 41 | 47 | 253 |
| | 42 | 59 | 410 | 507 |
| | 22 | 58 | 135 | ? |
| | 6 | 22 | 32 | ? |
| | 10 | 7 | 600 | 1,000 |
| | 6 | 304 | 257 | 263 |
| | 4 | 215 | 202 | 151 |
| *Sarcina lutea,* culture 96+, heated at 70° C. for 30 minutes | 30 | 1,000 | ? | 18,000 |
| | 15 | 15 | 3,000 | 24,600 |
| | 15 | 157 | 2,600 | 37,300 |
| | 14 | 11 | 3,200 | 26,700 |
| | 31 | ? | 2,100 | 32,100 |
| | 21 | ? | 3,500 | 18,800 |
| | ? | 72 | 2,000 | 12,700 |
| | ? | 126 | 3,500 | 26,700 |
| | ? | 700 | 4,000 | 15,300 |
| | ? | 341 | 5,000 | 17,800 |

* All cultures were 36-hour-old growth in 23.1 percent sucrose broth.
? = Contaminated plates.

others. After heating the suspensions were plated with nutrient agar containing 1 percent sucrose. The plates were incubated for two days at 37° C. The number of colonies developing on the agar plates after heating the suspensions of bacteria (table 4) indicated that as the concentration of the sugar was increased the number of bacteria surviving also increased. The concentration at which the number of the cells surviving begins to decrease was not determined.

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Tables 5, 6, 7 and 8 set forth the results in terms of numbers of tubes exhibiting growth after heating cultures of *E. coli, Micrococcus aureus, Streptococcus thermophilus* and *Sarcina lutea* respectively at different temperatures in broth containing 1, 23.1, 33.3, 41.2 and 50. percents of sucrose. In this trial, the respective cultures were grown in nutrient broth containing 23.1 percent sucrose for 36 hours. The cultures were then neutralized with 0.1 normal sodium hydroxid. Five c. c. portions were transferred to 35 c. c. portions of nutrient broth containing the desired percentages of sucrose. At least ten Sternberg bulbs were filled with approximately one c. c. of sucrose of the suspension and heated as desired. After heating the tubes were cooled in running tap water for 10 minutes and then the contents of the bulbs were forced by gentle heating into the tubes containing 1 percent sucrose broth. Growth was determined by changes in the hydrogen-ion con-

TABLE 5—*Escherichia coli*

| | Nutrient broth containing the following percentages of sucrose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 percent | | 23.1 percent | | 33.3 percent | | 41.2 percent | | 50 percent | |
| Tem-peratures used —°C. | Number of trials | Number of sur-vivals | Number of trials | Number of sur-vivals | Number of trials | Number of sur-vivals | Number of trials | Number of sur-vivals | Number of trials | Num of su vival |
| 62 | 10 | 8 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 63 | 10 | 3 | 10 | 8 | 10 | 8 | 10 | 9 | 10 | 10 |
| 64 | 10 | 0 | 10 | 3 | 10 | 8 | 10 | 5 | 10 | 7 |
| 65 | 10 | 0 | 10 | 0 | 10 | 4 | 10 | 4 | 10 | 6 |
| 66 | 10 | 0 | 10 | 1 | 10 | 1 | 10 | 2 | 10 | 2 |
| 67 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 1 | 10 | 0 |
| 68 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 1 |
| 69 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |

TABLE 6—*Micrococcus aureus*

| | Nutrient broth containing the following percentages of sucrose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 percent | | 23.1 percent | | 33.3 percent | | 41.2 percent | | 50 percent | |
| Tem-peratures used —°C. | Number of trials | Number of sur-vivals | Number of trials | Number of sur-vivals | Number of trials | Number of sur-vivals | Number of trials | Number of sur-vivals | Number of trials | Num of su vival |
| 66 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 67 | 10 | 9 | 10 | 8 | 10 | 10 | 10 | 10 | 10 | 10 |
| 68 | 10 | 6 | 10 | 8 | 10 | 7 | 10 | 9 | 10 | 10 |
| 69 | 10 | 2 | 10 | 5 | 10 | 6 | 10 | 5 | 10 | 7 |
| 70 | 10 | 0 | 10 | 2 | 10 | 3 | 10 | 4 | 10 | 6 |
| 71 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 1 | 10 | 2 |
| 72 | 10 | 0 | 10 | 0 | 10 | 2 | 10 | 0 | 10 | 2 |
| 73 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |
| 74 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |

TABLE 7—*Streptococcus thermophilus*

| Temperatures used —°C. | Nutrient broth containing the following percentages of sucrose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 percent | | 23.1 percent | | 33.3 percent | | 41.2 percent | | 50 percent | |
| | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Numb of survivals |
| 66 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 67 | 10 | 7 | 10 | 6 | 10 | 8 | 10 | 8 | 10 | 10 |
| 68 | 10 | 4 | 10 | 2 | 10 | 4 | 10 | 6 | 10 | 9 |
| 69 | 10 | 1 | 10 | 2 | 10 | 2 | 10 | 2 | 10 | 6 |
| 70 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 2 | 10 | 2 |
| 71 | 10 | 0 | 10 | 1 | 10 | 1 | 10 | 0 | 10 | 1 |
| 72 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 1 | 10 | 1 |
| 73 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 1 | 10 | 1 |
| 74 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |

TABLE 8—*Sarcina lutea*

| Temperatures used —°C. | Nutrient broth containing the following percentages of sucrose | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 percent | | 23.1 percent | | 33.3 percent | | 41.2 percent | | 50 percent | |
| | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Numb of survivals |
| 70 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 71 | 10 | 8 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 72 | 10 | 3 | 10 | 8 | 10 | 8 | 10 | 10 | 10 | 10 |
| 73 | 10 | 1 | 10 | 6 | 10 | 7 | 10 | 9 | 10 | 10 |
| 74 | 10 | 0 | 10 | 3 | 10 | 2 | 10 | 7 | 10 | 7 |
| 75 | 10 | 0 | 10 | 0 | 10 | 2 | 10 | 6 | 10 | 8 |
| 76 | 10 | 0 | 10 | 1 | 10 | 0 | 10 | 3 | 10 | 4 |
| 77 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 1 | 10 | 2 |
| 78 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 1 |
| 79 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |
| 80 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 | 10 | 0 |

centration, 0.1 percent of a 0.04 percent alcoholic solution of bromthymol blue having been added to the sucrose broths before sterilization.

The results reveal that as the temperatures are increased a gradual reduction occurs in the percentage of tubes containing surviving cells. As the concentrations of sucrose are increased the percentage of tubes showing survival increases.

Microscopic examination of the bacterial cultures in the sugar broths revealed the presence of capsules at certain stages during their growth. The writer feels that the capsule may be one of the factors (a portion of the cell wall) which aids in retarding the passage of moisture to the albumin within the cell. Because greater survival was obtained with higher concentrations of the sucrose, it appears that the sucrose itself does possess some protective action.

The theory that the protective action of sucrose depends on the lower moisture content of the protoplasm is supported to some degree by the observations of Bartlett and Kinne (1913) who have heated

spores in glycerin, olive oil, cottonseed oil, paraffin and water. The thermal death point was lower in water than in any of the other substances. The oils being non-aqueous could not dissolve the albumin, consequently the thermal death point was dependent on the amount of water originally distributed through the albumin within the cell. Glycerin, in spite of its toxicity, probably exerted merely a dehydrating influence. Bullock (1913), Seiffert (1914) and Dreyer and Walker (1912-13) have revealed similar facts. The above solutions do not ionize, or at most ionize to a very limited extent, while glycerin is the only one which possesses germicidal properties. More recent work (De Graaff, 1920) has revealed that certain oils and fats possess germicidal properties for some bacteria but not for others. The ligatures and sutures for surgical operations are sterilized in glycerin. Fenger, Cram and Rudnick (1920) have isolated two cultures of micrococci (probably sarcinae) and three sporeformers from supposedly sterile ligatures and sutures.

The observations of Viljoen (1926) on the protective influence of sodium chlorid for bacteria against heat are suggestive. Although the sodium chlorid may tend to dehydrate the cell the toxicity of the dissociated sodium and chlorin ions, in concentrations above 4 percent, probably is greater than the protective action of dehydration. As the temperatures are increased the activity of the dissociated ions and the undissociated molecules become correspondingly greater.

Magoon and Culpepper (1921) in connection with their studies on the penetration of heat in canning operations stated that one of the factors influencing the rate of penetration was the sugar and starch content of the canned substance. Viscous protein or pectin solutions were found also to retard the rate of change of temperature. However, where the time during which the bacterial cells are exposed to the desired temperature is sufficiently long for the entire contents to come to the temperature of the heating liquid, then the retardation in the rate of cooling is theoretically influenced in the opposite direction to the same degree.

### Influence of the Heating Medium on Heat Resistance

That the thermal death point of bacterial cells differs depending upon the solutions in which they are suspended when heated, has been established by Smith (1899), Russell and Hastings (1901, 1902), Zelenski (1906), Peiser (1915), Brown and Peiser (1916), and others. Von Esmarch (1889) was among the first to show that the resistance

of spores was dependent upon the nutrient medium used. Smith first observed that the thermal death point of the tubercle organism was higher in milk than in broth and in milk that was pasteurized in contact with the air it was still higher than that pasteurized in a closed vessel. On the surface of milk when pasteurized in contact with air a film is formed which protects the living cells from the action of the heat, the bacteria in the depths of the liquid having been killed.

Russell and Hastings (1902) confirmed the above observations and offered an explanation, as follows: Both the cooling effect of evaporation at the surface and the nature of the surface pellicle itself made possible the increased resistance of the cells. Cells incorporated in the surface film are more protected against the moisture in the surrounding medium than those in the depths of the liquid. Surface tension tends to form the film and during the film formation as well as afterwards the attraction of molecules for each other is greater at the surface than in the depths of the liquid.

Brown and Peiser (1916) suggested that the casein and fat offer some protection to bacteria when they are subjected to high temperatures. The average thermal death point among the cultures tested was in cream from 9° to 11° C., in whole milk 5° C., in separated milk 2.5° C., and in whey 0.5° C. higher than it is in bouillon. Salter (1919) observed that *E. coli* grew faster in the more concentrated peptone solutions. Orla-Jensen (1919) found certain species of bacteria (*Tetracocci*) frequently in sweetened condensed milk. Greig-Smith (1909) and Downs (1925) observed that *Micrococcus albus* is one of the common causes of coagulation in sweetened condensed milk. Apparently some of the non-sporeforming species are more adapted than others to endure the heat treatment under the conditions which exist during the processing of sweetened condensed milk. Holman (1914) found that albuminous fluids, such as milk, beef serum and ascites fluid, have a protective action for bacteria against heat.

Winslow and Falk (1923) found that either sodium chlorid or calcium chlorid, in suitable concentrations, may be stimulating as well as toxic to *E. coli* suspended in non-nutrient menstrua. Winslow and Brooke (1927) showed that salt and sugar solutions in the concentrations employed are no more favorable than distilled water to the bacteria studied. The species have been strains of *E. coli, B. megaterium, B. cereus* and *Serratia marcescens* (*B. prodigiosus*). A menstrum containing one part of nutrient broth in 100 parts of water completely destroys the lethal effect and still higher dilutions reduce it materially. They concluded that the influence of the meat extract and of peptone

in thus promoting the viability of bacteria can not presumably be caused by their use as nutrients, that it can not be caused by osmotic effects and that it would seem probable that the phenomenon is analogous to the operation of a protective colloid. The endless amount of material which has been published from Winslow's laboratory on the viability of bacterial cells in distilled water containing known substances under controlled conditions clearly indicates how intricate the relations must be in solutions containing the great variety of substances which bacteriologists use as culture media. Although the use of distilled water may reduce certain complications to a minimum it introduces another, namely the influence of a hypotonic solution.

## ADAPTABILITY OF SPECIES TO HIGHER TEMPERATURES

The entire subject of variability of species will not be discussed. However since increased thermal resistance is one form of variability its possibilities must be considered. That great adaptability is a necessity for the continued existence of microorganisms, and of higher life to a lesser degree, will be recognized only when one considers the nature of the protoplasm itself and the inability of the lower species voluntarily to change their environment at the approach of adverse conditions. Variability, therefore, must be the result of an attempt on the part of a given species to adjust itself to new conditions.

### Nature of Protoplasm

Protoplasm assumes morphologic and physiologic changes as a result of a series of responses to stimuli. Protoplasm responds differently to different kinds of stimuli and different kinds of protoplasm in turn respond differently to the same stimuli. The acclimatization process may be defined as the phenomenon of increased resistance capacity acquired by the organism. This property may be individual or racial. If individual, the species becomes a variant; if racial, it becomes a mutant with possibilities of species significance. To distinguish between inherent and artificial stimuli is often difficult. One of the most common examples of racial adaptation, perhaps it is too common to be recognized by many bacteriologists, is spore formation.

### A Normal Habitat

What constitutes the natural habitat of any species varies with each species and the bacteriologist can not accurately state that any

species on artificial substrata has the identical morphology and physiology that it had previous to isolation. To define a normal habitat, free from all substances which might cause variability, is to attempt the impossible. Therefore, following the example of others, the writer assumes that nutrient broth or agar meets those requirements as perfectly as possible.

That morphological changes occur under what Koch would recognize as normal conditions has been established by Schierbeck (1900), Almquist (1916), Löhnis and Smith (1916), Clark and Ruehl (1919), Löhnis (1921), Bergstrand (1923a, 1923b, and 1926), Henrici (1921-1926b), Evans and Freeman (1926), and Mellon (1917-1927). Hadley (1927) recognized a bacteriophage-like agent which frequently caused variation in old cultures. Manifestations of physiological changes have been observed during normal growth by Schultz and Ritz (1910), Sherman and Albus (1923) and Oerskov (1925) who maintain that cells in the adolescent stage are more sensitive than mature cells. Morphological and physiological differences in the cells of young and old potato plants have been demonstrated by Lutman (1926). MacDougal (1926) reported a hardening and thickening of the cell walls of *Carnegiea gigantea* with age. That latent fermenting powers are often developed when cultures are grown on media containing a supposedly non-fermentable substance has been demonstrated by Massini (1907), Twort (1907), Burk (1908), Sauerbeck (1909), Kowalenko (1910), Jacobsen (1910), Müller (1911), Sorbernheim and Seligmann (1911), Thaysen (1911), Burri and Düggeli (1909), Burri (1910), Burri and Adrejew (1910), Bernhardt and Markoff (1912), Baerthlein (1912a, 1912b), and Grey (1924) and more recently reviewed by Dobell (1912-13) and Hadley (1927).

## Examples of Adaptability to Heat

The most remarkable record of acclimatization to heat was published by Dallinger (1887) who accustomed one of the flagellates during a period of several years until it withstood a temperature of 70° C. whereas at the outset it grew at 15.6° C. At the beginning of the experiment the protoplasm was killed at 23° C. Davenport and Castle (1895) held a portion of a batch of frogs eggs at 24° C. to 25° C. and the remainder of the batch at the usual temperature of 15° C. The point of heat rigor was 3.2° C. higher for the tadpoles which had been hatched at the higher temperature.

Gage and Stoughton (1906) attempted to develop a heat-resistant strain of *E. coli* by subculturing colonies of the most resistant cells which had grown on the agar plates. Instead of developing strains with greater heat resistance, they obtained strains whose thermal resistance more nearly approached that of the average. Magoon (1926) more recently developed a strain on *B. mycoides* by selection with greater resistance to heat than that of the parent strain.

Dozier (1924) and Starin (1926) found that the toxigenic powers of *Clostridium botulinum* could be altered by growing in different substrates and at different temperatures. Wadsworth and Kirkbride (1918) have reported similar observations for the virulence of the pneumococcus. Pasteur, Chamberland and Roux (1881) used heat to attenuate the anthrax bacillus. Drying has been used to attenuate the rabies virus in the early prophylactic treatment. Gibier (1882) found that frogs were susceptible to anthrax when the body temperature was 35° C. but not when it was 20° C.

Both drying and heating, therefore, are recognized as agents which produce adaptations and consequently modifications in the life of the species.

Similar observations have been recorded of the responses of living protoplasm to other stimuli but it is not the purpose of this investigation to review these records of adaptation.

## The Normal Existence of Life at High Temperature

To offer an explanation of the active growth of thermophiles in hot liquids is far more difficult. Davenport and Castle (1895), whose statements will be quoted in part, argue that this adaptation is a natural consequence of environment. Dallinger (1887) in his experiments on the accustoming of the *Flaggellata* to higher temperatures, observed changes in the vacuolization of the organisms accompanying the elevation in temperature. Thus he found that when all the flagellates had remained at one of the stationary points for some time, vacuoles appeared in the protoplasm and then disappeared and at the same time with their elimination from the protoplasm it gained the capacity of resisting the higher temperature. These observations suggest that even the active organism must get rid of water from the protoplasm before its protoplasm can resist the higher temperature. The authors next ascertained if acclimatization to higher temperatures did not depend upon the reduction of the amount of water in the chylema spaces—the

organisms in this respect becoming more like a spore in which the quantity of free water is reduced to the minimum.

The increased excretion of the water from the protoplasm which occurs when the latter is subjected to high temperatures seems to be connected with the increased katabolism accompanying high temperatures. Rossbach (1872) has demonstrated that the rate of formation of the contractile vacuole in *Ciliata* increases with the temperature. That the excretion of fluids by land mammals is extraordinarily abundant in hot air needs no demonstration.

If then, organisms become acclimatized to higher temperatures by losing water from their protoplasm, and if the loss of water is a necessary consequence of the increased chemical activity resulting from warmth, it follows that individual acclimatization does occur and must occur as a necessary concomitant of increased temperature.

From this point of view the resistance of the organisms living in hot springs is not something to wonder at, but is the necessary result of their environment. A note at the close of Davenport and Castle's paper states that J. I. Hamaker (a worker in the laboratory) acclimatized certain *Ciliata* to dense solutions, by which operation the body became smaller and the protoplasm denser. Owing to the fact that water was not imbibed by the protoplasm as readily as when the organism was immersed in pure water, growth was thus inhibited. In these *Ciliata,* as the amount of chylema was reduced the point at which heat rigor occurs was elevated.

## SUMMARY

The literature on the causes of thermal resistance has been reviewed. The thermal resistance of microorganisms seems to depend in a preponderating degree on the moisture content of the cell.

The cell wall of the spore is less permeable to water than that of the vegetative cell. The spore itself has a lower moisture content.

Cells subjected to desiccation survive longer when encapsulated than when not encapsulated. The capsule retards the loss of moisture. Sarcinæ survive desiccation better than some of the other species which have been under observation. Sarcinæ survive higher temperatures than the micrococci and the common non-sporeforming rods. *Microbacterium lacticum,* Orla-Jensen (1919) and *Lactobacillus thermophilus,* Ayers and Johnson (1924) are exceptions to this rule.

Hypotonic solutions as indicated by comparisons of heating cells in distilled water and in physiological salt solutions, decrease the heat resistance of the cells. The greater death rate may be explained, in

part, by the lower osmotic pressure of the distilled water outside of the cell wall.

Hypertonic solutions, as indicated by comparisons of heating cells in nutrient broths containing increasing concentrations of sucrose, have a protective action up to and including 50 percent sucrose. The protective action may be explained, in part, by the dehydrating (osmotic) influence of the concentrated sucrose solution outside of the cell wall and, in part, by the presence of capsules formed about the cells when grown in the sucrose solution before the determinations were made.

The thermal death points of microorganisms vary depending on the medium in which the determinations are made.

The literature suggests that certain microorganisms when gradually subjected to increasing temperatures have a faculty of adaptability. The process of adaptability depends on the elimination of water from the cell contents.

Similar forces which tend to produce spores for the sporeforming species are probably active to a lesser degree in non-sporeforming species when they are subjected to high temperatures or other adverse conditions.

## CONCLUSIONS

The ability of living protoplasm to survive high temperatures depends in part, at least, on its low moisture content. Data are presented and literature cited which suggest that: (1) changes in the nature of the cell-wall membrane, (2) changes in the cell produced by growth in concentrated sucrose solutions and (3) changes evolved through acclimatization processes may be instrumental in producing a cell with a low moisture content and consequently a higher thermal resistance.

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# THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK

## III. DESCRIPTION OF THE NON-SPORE-FORMING, THERMODURIC ORGANISMS ISOLATED

A. H. ROBERTSON

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# THERMOPHILIC AND THERMODURIC MICRO-ORGANISMS WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK

## III. DESCRIPTION OF THE NON-SPORE-FORMING, THERMODURIC ORGANISMS ISOLATED[1]

A. H. ROBERTSON[2]


### ABSTRACT

The present study is an outgrowth of an attempt to determine one or more of the bacterial species which may cause the so-called "pin-point" colonies on pasteurized milk plates.

Cultures of non-spore-forming, thermoduric bacteria have been secured from the following sources: (1) Pasteurized milk plates containing "pin-point" colonies from several cities in this country, (2) milk which had been incubated at pasteurizing temperatures for several hours, (3) milking machines which had been improperly washed and sterilized, and (4) accidental contaminations.

Cultures in which 90 per cent or more of the cells survived pasteurization were retained for further study. These cultures belong to the following five species named in the order of their resistance to heat: *Microbacterium lacticum* Orla-Jensen, *Sarcina lutea* Schröter, *Streptococcus thermophilus* Orla-Jensen, *Sarcina rosea* Schröter, and *Micrococcus conglomeratus* Migula. *Microbacterium lacticum* and *Streptococcus thermophilus* may form "pin-point" colonies on agar plates.

The presence of the above species in market milk is not regarded as dangerous to health.


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[1]Portion of a thesis submitted to the Graduate School at Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy. The entire contribution appears in four parts, two of which are Technical Bulletins Nos. 130 and 131 of the New York State Agricultural Experiment Station and two Bulletins Nos. 274 and 275 of the Vermont Agricultural Experiment Station. The author acknowledges the many helpful suggestions offered during the course of the present investigation by Dr. R. S. Breed of the New York Station and by Dr. H. B. Ellenberger and Dr. B. F. Lutman of the Vermont Station.

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## INTRODUCTION

A large part of the milk consumed in the cities of this country is pasteurized. Since the World War the unusual sporadic appearance of large numbers of small, punctiform, white bacterial colonies has been observed on agar plates prepared from pasteurized milk in some of these cities. At first these were attributed to unsanitary practices in the pasteurizing plants, but controlled experiments revealed that certain bacteria grew during pasteurization. During pasteurization, several samples of the pasteurized milk might be taken at intervals and the bacterial counts of these samples would be normal at first, i.e., under 30,000 colonies per cc, when suddenly there would be an increase to 200,000 per cc, or even more. Dotterrer (1923)[3] compared samples taken in this way with a series of samples taken approximately 30 minutes before the pasteurized sample was secured. The counts of the raw milk samples indicated that the increase was not necessarily associated with an increase in the bacterial content of the milk in the supply tank.

The flora in question appears and disappears sporadically in some plants and is an addition to the flora of pasteurized milk which has been earlier observed. Since many cities have ordinances limiting the bacterial content of both raw and pasteurized milk, a further study of the thermophilic and thermoduric, non-spore-forming bacteria of pasteurized milk has been made. In this report, a 90 per cent survival as determined by the agar plate method was required of any bacterial species before it was regarded as heat-resistant. Therefore the species which are given extended study had a "majority thermal death point" above the pasteurization temperatures used.

## HISTORICAL

The literature on the presence of non-spore-forming bacteria in pasteurized milk is not extensive. The related work on the survival of bacteria when exposed to heat can not properly be omitted. Probably Ayers and Johnson (1913) have contributed the most comprehensive study of pasteurization methods and temperatures with special reference to their influence upon the flora observed. The authors have observed some of the fermentative powers of a large number of cultures and divide the species into five groups, the weak acid-forming types, the acid and coagulating, the inert, the al-

[3]Refers to Literature Cited, page 54.

kali-forming, and the peptonizing types, according to their action in litmus milk. Pasteurization increased the percentage of the acid-forming group in milk, while the other groups decreased. The average percentage of lactic acid bacteria in raw milk which survived pasteurization for 30 minutes at 62.8°C ranged from 1.27 to 4.55 in the various grades of milk. Pasteurization at temperatures above 76.7°C for three minutes largely destroys the acid-forming bacteria and the spores of the peptonizing types remain alive. For the purpose of comparison, the types surviving 62.8°C for 30 minutes will be discussed later (page 22) in association with the isolations made in the present investigation.

Sternberg (1887) was among the first to observe the thermal death points of some of the pathogenic bacteria. Some of the species of micrococci were able to resist 62°C for 10 minutes and 60° C for 30 minutes. Hauser (1887) also found certain of the micrococci to be more resistant to heat than others. Adametz (1890) found certain species of sarcinae in both raw and sterilized milk.[4] Van Geuns (1889) heated pure cultures of white and yellow micrococci, white, green, and gray short rods, and white, gray, orange, red, and violet non-spore-forming long rods at 80° C for 90 seconds. After heating the cultures were cooled to 12° C. Two cultures of white micrococci, a translucent, gray, non-liquefying, short rod and a white liquefying, long rod, survived. He found a chain-forming micrococcus in slime which survived 70° C for 90 seconds. Van Geuns (1889) and de Man (1893) reported that the non-spore-forming pathogenic species were usually killed by exposure to 60° C for 90 seconds.

Dyar (1895) isolated a yellow, non-liquefying, nitrate-reducing micrococcus (probably a sarcina) from a jar of sterilized milk which was heavily seeded with this species. After growth in milk for six days, the milk was coagulated on boiling. Stubenrath (1897) found the sarcinae to be unusually resistant to heat. Marshall (1897) was perhaps the first to report the characteristic lactic acid fermentation in pasteurized milk similar to that occurring in raw milk. Weiss (1899) isolated three lactic acid species from mash, the optimum growth temperatures of which are between 40° and 45° C.

Smith (1899) showed that the surface layer of pasteurized milk has a protective action on the tubercle bacilli. Hashimoto (1901) described two lactic acid, coccus forms (a micrococcus and a strepto-

[4]Term used to signify practical sterilization.

coccus) which were isolated from sterilized milk. Russell and Hastings (1901, 1902a) confirmed the observations of Smith (1899) by proving that bacteria in the scalded layer surface pellicle of milk heated in contact with air survive, while bacteria in the depths of the liquid are killed. Both the cooling effect of evaporation and the nature of the scalded layer itself produce the increased resistance of bacteria in milk pasteurized in contact with the air. The scalded layer has a lower moisture content and under these conditions heat rigor is retarded.

Russell and Hastings (1902b) describe a Gram-positive, non-liquefying, bright lemon-yellow micrococcus, the thermal death point of which is 76° C in milk. The authors assume that a different kind of protoplasm explains the unusual heat resistance of this micrococcus. Freudenreich and Thöni (1904) studied a number of highly heat-resistant lactic acid bacteria. They reported that *Bacterium lactis acidi* Leichmann (1896) survived 60° C for 30 minutes, 65° C for 15 minutes, and 70° C for 5 minutes. Rogers (1905) found lactic acid bacteria in milk pasteurized at 85° C by the flash method and held at 20° C, which occasionally, after 24 hours, completely inhibited the peptonizing bacteria. Henneberg (1906), attempting to prevent the growth of undesirable yeasts and bacteria in malt, found that a culture of the lactic acid bacterium, *Bacillus delbrücki* Leichmann (1896), was killed at 65° C in five minutes and at 72.5° C in five minutes when in a concentrated mass.

De Jong and de Graaff (1906) isolated a strain of *Escherichia coli* Castellani and Chalmers which withstood 85°C. Frequently it survived 65° to 66°C for 30 minutes. The culture was killed more easily in bouillon than in milk. On one occasion this culture survived 70° to 72°C for 30 minutes. The authors, therefore, recommend a temperature of 70°C for a period of 30 minutes to kill pathogens. Zelenski (1906) found a strain of *Escherichia coli* which survived unusually high temperatures. Gage and Stoughton (1906) showed that the majority thermal death point of *Escherichia coli* was 50° to 60°C. Attempts to reproduce and develop a progeny with greater heat resistance from colonies that survive the higher temperatures failed. Adelloff (1907) reported that some of the lactic acid bacteria survive pasteurization temperatures.

Wolff (1908) pasteurized milk at 70°C and found that some of the lactic acid bacteria survived. The percentage survival depended to some degree on the amount of acid present which aided in the for-

mation of a protective covering of casein and albumin about the cells so that they could not only resist the higher temperatures but also carry other organisms along with them.

Eijkmann (1908) first discussed the shape of the rate-of-killing curve for bacterial cultures when exposed to heat. The curve assumes a reversed "S" appearance, showing that a few cells are killed at first, then a majority are killed suddenly, followed by a few possessing an unusually high heat resistance. Rosenau (1908) confirmed the earlier work of Sternberg (1887), Van Geuns (1889), and de Man (1893) in which they have studied the thermal death points of some of the pathogens.

Greig-Smith (1909) and Downs (1925) associated *Micrococcus albus* Buchanan with the coagulation of canned condensed milk. Ayers and Johnson (1910) proved that lactic acid bacteria of high thermal death point are frequently found in milk. In milk pasteurized at 60°C for 30 minutes, the percentage of acid-forming colonies that survived (as determined by plating) ranged from 0.001 to 18.91, the average being 4.8 per cent of the total acid colonies. As the temperature used is increased, the percentage surviving is gradually decreased so that when 65.6°C is employed for 30 minutes the average survival is only 0.74 per cent of the total number. The thermal death point of one lactic acid organism which was isolated from milk is 74.4°C in broth and 75.6°C in milk when heated in Sternberg bulbs for 30 minutes. When heated ten minutes the thermal death point is 77.8°C. In pasteurized milk and in clean raw milk, the relative proportions of the groups of bacteria, i.e., acid forming, alkali or inert forms, and the peptonizing types, are approximately the same. The alkali or inert forms are in the majority in both cases. They increase in proportion during the first day and then gradually decrease until the fifth day when the proportion is lower than that of the lactic acid group. The acid group constitutes between 20 and 30 per cent of the total and after a decrease during the first 20 hours gradually increases to form the largest proportion on the sixth day. The proportion of the peptonizing types increases during the first 24 hours and then gradually decreases.

Lingelsheim (1912) reported that *Streptococcus brevis* withstood 65°C for 20 minutes, 70°C for 10 minutes, and 80°C for 5 minutes. Ayers and Johnson (1915) discovered that the thermal death point of 174 cultures of the colon bacillus isolated from cow feces, milk, cream, human feces, flies, and cheese varied considerably

when heated in milk for 30 minutes under conditions similar to pasteurization. At 60°C, the lowest pasteurization temperature used, 95 cultures (54.59 per cent) survived and at 62.8°C, 12 (6.89 per cent) survived. One culture was not killed at 65.6° C on one occasion only. On all subsequent trials it was killed at this temperature. All cultures had low majority death points and survived on account of a few cells only. Holman (1914) found that certain albuminous liquids possessed a protective action for bacteria against heat. Gorini (1915) believed that the resistance of non-spore-forming bacteria depended on the formation of a protective coating of casein around the bacteria, the latter being responsible for the coating. Peiser (1915) pointed out that the protective action given to the suspended lactic acid bacteria in separated milk by the casein and coagulated albumin raises their thermal death point 2.5°C over that in bouillon and that the protein and fat of whole milk raises it 5.0°C. Weigmann, Wolff, Trensch, and Steffen (1916) report that the lactic acid bacteria are in larger proportions in the pasteurized milk than in the same milk before pasteurization. Souring is retarded in the pasteurized milk, altho the proportion of the acid bacteria is greater.

Patzschke (1916) isolated a *Streptococcus lactis thermophilus* which was killed after a temperature of 85°C had been maintained for from 10 to 20 minutes. Brown and Peiser (1916) concluded that casein and fat in milk offered some protection to bacteria when the latter are subjected to high temperatures. Ayers, Johnson, and Davis (1918), working with 35 strains of pathogenic streptococci, found 8 strains which survived 140°C for 30 minutes. According to their other cultural characters these eight strains would not be classed as pathogenic. As the pH varied from neutrality, the thermal death rate increased. Twiss (1920) reported that some of the members of the para-typhoid group survived pasteurization temperatures. Krumweide and Noble (1921) pointed out that some error had been introduced into Twiss's work as they were unable to obtain the same results. Gorini (1921) reported that the non-spore-forming lactic acid bacteria are killed if exposed for 15 minutes at 60° to 80° C. One case is mentioned where the thermal death point was 100° C. In the case of the acid-rennet-forming coccus, it is completely enveloped in a covering of casein.

Orla-Jensen (1919, 1921) discussed extensively the thermal resistance and the growth relationships to temperature of the lactic acid

bacteria, among which are *Streptococcus cremoris, S. thermophilus, S. lactis, Microbacterium lacticum, Thermobacterium bulgaricum, T. helveticum,* and certain sarcinae. Göbel (1923) found that the resistance to heat of the individual cell was greater in a dilute suspension than in a more concentrated suspension of the cells. Bleyer (1923) succeeded in recovering cultures of streptococci from the liver, spleen, and heart-blood of rats after their death. Prior to inoculation the streptococci had been subjected to temperatures of 50°, 56°, 60°, 65°, 70°, and 80° C for one hour in a water bath. Behrens (1923) concluded that there was a certain dilution of the bacterial suspension where the optimum resistance to heat was secured. A concentration greater or less than this resulted in a higher percentage of killing of the cells. Behrens worked with *Escherichia coli* and *Bacillus pyocyaneus* Gessard (1882). Fleischer and Amster (1923) have shown that the nature of the medium influences the thermal death points of the bacteria.

Harding (1923) reported daily fluctuations in bacterial counts during a day's run at large pasteurizing plants that indicate the presence of thermoduric bacteria. Ayers and Johnson (1923) pointed out that it would be unwise to attempt to transport milk in a hot condition because of the development of a thermophilic flora, some of which grow rapidly. Yates (1923), without considering the possibility of thermophilic bacteria, thought that the reaction of the media or the use of chlorine solutions was partly responsible for the presence of pin-point colonies on the agar plates. Robertson (1923), in a preliminary report on some non-spore-forming heat-resistant bacteria in pasteurized milk, isolated relatively large numbers of spherical bacteria, a few rods, and some species resembling *Streptococcus lactis* Löhnis. Yates and Glover (1923) found that the count of pasteurized milk at the end of a long run was greater than that of the raw milk as it left the receiving vat. Van Horn (1924) obtained about three times as high counts with the Difco beef extract agar as with the pre-war Leibig's beef extract agar when plating pasteurized milk. When plating raw milk the counts were only slightly higher on the Difco product.

Taylor, A. R. (1924) found that the milk leaving the holder was occasionally from 5.6 to 10.8 times the count of the same raw milk as it entered the preheater. Taylor, G. B. (1924) recognized two types of bacteria, one which was merely resistant to pasteurizing temperatures and one which grew rapidly at these temperatures. Hunger-

ford and Harding (1924) held samples of milk at the pasteurizing temperatures and found increased bacterial counts at the end of six hours. Tanner (1924a), using an enrichment method to make the thermophiles more abundant, isolated a number from milk. Adams and Harding (1924) secured thermophiles from 28.2 per cent of samples of common raw milk, 43.7 per cent of the Class "A" raw milk, and 40.4 per cent of the certified milk. Harding and Ward (1924) obtained samples from the storage vats of large city pasteurizing plants and found that by incubation at pasteurizing temperatures up to four and six hours a thermophilic flora could be developed.

Cooledge (1924) suggested the use of a medium with a pH of 7.2 for the growth of the non-spore-forming thermophilic flora. Morrison and Tanner (1922) isolated two cultures from a bottle of raw milk of excellent quality. Ayers and Johnson (1924a) pointed out the significance of the terms "majority" and "absolute" thermal death points of bacteria. Ayers and Johnson (1924b) described the organism causing the "pin-point" colonies and warned against the too broad use of the term "pin-point." The pH of the media and the crowded plates often give a plate characteristic of the "pin-point" colony condition. The causal organism was named *Lactobacillus thermophilus*. Fay (1927) has warned against the unrestricted use of the term "pin-point" colony as it superficially characterized the colony growth of several species of bacteria on the agar plates. Many of the common mesophiles, and some of the thermoduric flora and some of the true thermophilic species may form "pin-point" colonies. The writer has observed spore-forming thermophiles whose colonies also might be considered "pin-point" in size.

Johnson and Exworthy (1925) observed a streptococcus associated with abnormally high plate counts of pasteurized milk supplies. The organism could not be traced to improper pasteurization nor to unsanitary dairies. Swenarton (1925, 1926) associated the presence of "pin-point" colonies with milk produced in the spring. Fifty of the 52 cultures isolated were streptococci. No data are presented regarding their heat resistance. Tanner and H. G. Harding (1926a, 1926b) demonstrated thermophilic bacteria in all samples of milk after the milk had left the barn. The 73 cultures were Gram-positive, motile, spore-forming rods which grew well at pasteurizing temperatures. Several were strict thermophiles growing only above 37° C. More recently, Clarke and Dougherty (1926) have confirmed some of the observations on spore-forming and non-spore-forming ther-

mophilic species. Fay (1926, 1927) has described three types of thermoduric saccharophilic organisms which form characteristic "pin-point" colonies. These are probably members of the lactic acid group. Jenkins (1926) has found that certain common non-spore-forming species survive pasteurization temperatures also.

H. G. Harding (1927) has discussed the practical application of the facts which have recently been discovered regarding the occurrence and significance of thermoduric and thermophilic bacteria in milk.

## SOURCE OF MATERIAL

Frequent reports of the presence of "pin-point" colonies of bacteria on milk control, agar plates were made at the Cleveland Meeting of the American Public Health Association (1923). This led the Committee on Standard Methods of the Laboratory Section to ask the Referee on Bacteriological Methods of Milk Analysis to make a study of the occurrence of these bacteria. Plates from Kansas City (Mo.), Detroit, Chicago, Milwaukee, Jackson (Mich.), Boston, and Baltimore were sent to the New York State Agricultural Experiment Station at Geneva. A total of 465 cultures were obtained from representative colonies on the pasteurized milk plates. In some cases where the probable growth of the colony was doubtful, a portion of the agar on which the colony was growing was put into the sterile milk tube.

Since only cultures that were resistant to pasteurizing temperatures were desired for further study, milk cultures grown at 25° C less than 24 hours old were exposed in Sternberg bulbs to a pasteurizing temperature. In this work, unless otherwise stated, a pasteurizing temperature of 145° F (62.8° C) for 30 minutes was used. The bulbs were immediately cooled to 40° F or less for 20 minutes and then the milk was plated with nutrient agar containing 1 per cent d-glucose and 0.1 per cent of a 0.001 per cent alcoholic solution of brom cresol purple. Only such plates as had a large number of colonies after incubation at 25° C for five days were recorded as resistant to pasteurizing temperatures. A summary of these results appear in Table 1.

In addition to the cultures secured in this way, Dr. D. H. Bergey of the University of Pennsylvania sent ten cultures of heat-resistant micrococci which he had isolated from milk. In the table, these are indicated by their source, Philadelphia.

By means of enrichment methods, the author isolated 132 cultures from the market milk supply of Geneva, N. Y., in 1925 and 41 from

the supply of Burlington, Vt., in 1927. These heat-resistant cultures were obtained by incubating samples from individual producers at

TABLE 1.—SOURCES OF THE 465 CULTURES FROM PASTEURIZED MILK PLATES.

| DATE OF RECEIVING PLATES | PLATES SENT FROM | PLATE No. | NUMBER OF ISOLATIONS FROM PLATE | NUMBER OF CULTURES SURVIVING PASTEURIZATION |
|---|---|---|---|---|
| Nov. 6, '22 | Kansas City | 1 | 15 | 15 |
| Nov. 16, '22 | Jackson, Mich. | 2 | 7 | 4 |
| Nov. 6, '22 | Kansas City | 3 | 14 | 10 |
| Nov. 6, '22 | Kansas City | 4 | 14 | 2 |
| Dec. 26, '22 | Kansas City | 5 | 6 | 1 |
| Jan. 15, '23 | Philadelphia | * | 10 | 7 |
| Dec. 26, '22 | Kansas City | 6 | 10 | 4 |
| Dec. 26, '22 | Kansas City | 7 | 9 | 0 |
| Nov. 28, '22 | Kansas City | 8 | 18 | 0 |
| Jan. 26, '23 | Chicago | 9 | 9 | 2 |
| Jan. 26, '23 | Chicago | 10 | 12 | 2 |
| Jan. 26, '23 | Chicago | 11 | 13 | 6 |
| Feb. 1, '23 | Detroit | 12 | 11 | 2 |
| Feb. 1, '23 | Detroit | 13 | 12 | 2 |
| Jan. 19, '23 | Detroit | 14 | 3 | 1 |
| Jan. 19, '23 | Detroit | 15 | 3 | 0 |
| Feb. 1, '23 | Detroit | 16 | 9 | 1 |
| Feb. 1, '23 | Detroit | 17 | 8 | 1 |
| Feb. 1, '23 | Detroit | 18 | 9 | 4 |
| Feb. 1, '23 | Detroit | 19 | 7 | 1 |
| Dec. 26, '22 | Kansas City | 20 | 6 | 1 |
| Feb. 1, '23 | Detroit | 21 | 12 | 0 |
| Feb. 16, '23 | Boston | 22 | 9 | 9 |
| Feb. 16, '23 | Boston | 23 | 55 | 0 |
| Feb. 16, '23 | Boston | 24 | 3 | 1 |
| Jan. 23, '23 | Milwaukee | 25 | 13 | 6 |
| Feb. 14, '23 | Kansas City | 26 | 12 | 9 |
| Feb. 14, '23 | Kansas City | 27 | 18 | 14 |
| Feb. 14, '23 | Kansas City | 28 | 18 | 13 |
| Feb. 14, '23 | Kansas City | 29 | 7 | 3 |
| Feb. 14, '23 | Kansas City | 30 | 6 | 6 |
| Dec. 26, '22 | Kansas City | 31 | 4 | 2 |
| Feb. 14, '23 | Kansas City | 32 | 10 | 2 |
| Feb. 21, '23 | Detroit | 33 | 2 | 1 |
| March 2, '23 | Kansas City | 34 | 6 | 0 |
| March 2, '23 | Kansas City | 35 | 5 | 4 |
| March 2, '23 | Kansas City | 36 | 6 | 4 |
| March 2, '23 | Kansas City | 37 | 5 | 4 |
| March 20, '23 | Baltimore | 38 | 8 | 1 |
| March 20, '23 | Baltimore | 39 | 5 | 3 |
| March 20, '23 | Baltimore | 40 | 4 | 3 |
| March 20, '23 | Baltimore | 41 | 6 | 0 |
| March 20, '23 | Baltimore | 42 | 12 | 11 |
| March 16, '23 | Chicago | 43 | 5 | 5 |
| March 16, '23 | Chicago | 44 | 4 | 4 |
| March 16, '23 | Chicago | 45 | 4 | 4 |
| March 16, '23 | Chicago | 46 | 5 | 5 |
| March 16, '23 | Chicago | 47 | 7 | 7 |
| March 16, '23 | Chicago | 48 | 9 | 9 |

*Isolated cultures were sent from Philadelphia.

145° F for a period of seven hours. Plates were prepared hourly and the counts obtained as shown in Table 2. Four of the 30 Geneva samples and 1 of the 30 Burlington samples showed an increase in the count during the holding period. The colonies of the types which increased in numbers were small, round, and dull white. They might be described as "pin-point" colonies. An examination of the cultures made from representative colonies revealed that of the 132 Geneva cultures and of the 41 Burlington cultures, 97 and 34, respectively, were rods representing the same species. The remaining cultures were either yellow or white cocci with the yellow ones predominating. The data on which these conclusions are based will be discussed later (page 28).

TABLE 2.—CHANGES IN THE PLATE COUNT OF SAMPLES OF MILK HELD AT PASTEURIZING TEMPERATURES.

| SAMPLE No. | TIME OF HOLDING | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 hours | 1 hour | 2 hours | 3 hours | 4 hours | 5 hours | 6 hours | 7 hours |
| | Geneva, N. Y., Milk Supply* | | | | | | | |
| 1 | 113,000 | 3,000 | 7,000 | 23,000 | 47,000 | 18,000 | 15,000 | 26,000 |
| 2 | 540,000 | 21,600 | 16,000 | 40,000 | 114,000 | 52,000 | 67,000 | 28,000 |
| 3 | 24,000 | 6,000 | 17,000 | 81,000 | 103,000 | 74,000 | 58,000 | 67,000 |
| 4 | 140,000 | 20,400 | 26,600 | 69,000 | 218,000 | 141,000 | 121,000 | 93,000 |
| | Burlington, Vt., Milk Supply† | | | | | | | |
| 1 | 193,000 | 51,000 | 17,600 | 44,000 | 151,000 | 167,000 | 123,000 | 88,000 |

*26 other samples tested but no growth attributable to non-spore-forming thermophiles appeared.

†29 other samples tested but no growth attributable to non-spore-forming thermophiles appeared.

By accident on two different occasions in an attempt to obtain thermophiles by incubating samples of milk at 145° F for three days, a culture of a yellow sarcina developed after the samples had stood at room temperature for an additional five days. Because of their peculiar heat resistance, these two cultures, Nos. 1Z and 2Z, have been included in this discussion among the cultures spoken of as Type III (page 49).

During the course of this work, samples of milk from various sources were frequently incubated at pasteurizing temperatures for several days at a time. Usually, spore-forming thermophiles developed. The difficulties of isolating non-spore-forming thermophiles are far greater than those encountered in the isolation of the spore-forming type.

H. G. Harding sent some pasteurized milk plates from Madison, Wis., to the Geneva laboratory in December, 1925. The material

was broken in transit so that no isolations could be made directly. Parts of the agar were placed in three sterile milk tubes and incubated at 25° C for a few hours. The milk tubes were then placed at pasteurizing temperatures for a period of five hours. Plates were prepared at the end of the third, fourth, and fifth hours. Practically a pure culture of a pink sarcina developed on all of the plates from each of the three inoculated tubes. Type V (page 50) consisted of 21 isolations made from these plates. Cultures H1 and H19 were retained for further study.

A total of 415 out of 721 milking machine cultures isolated in a previous investigation, Robertson (1924), were available and milk cultures of these were pasteurized. These results will be discussed in their proper sequence (pages 29 to 39).

The natural habitat of the cultures under observation cannot be determined accurately. Probably they represent either water, air, or soil species. Frequently they were found in practically pure cultures on the pasteurized milk plates. The isolations of the heat-resistant species from the Geneva milk supply suggest that they may be found in the milk of one out of every 7 or 8 producers, while those from Burlington indicate that they may be isolated from the milk of one out of every 30 producers.

## TECHNIC

The following technic has been used thruout this work. Observations were made as to the grouping of the cells both from the 24-hour-old milk cultures and from the growth on agar slants. The preparations from the agar slant growth were stained by the Gram method, while the milk preparations were made and stained according to the Breed method. Morphology was observed on successive days in some unusual cultures. The Gram stain was made with the Hucker (1921) ammonium oxalate method. Chromogenesis was determined on the two-weeks' old agar slant. Motility was studied in a culture less than 24 hours old.

The ability of the cultures to use carbohydrates and organic acids was determined by adding 1 per cent of the desired substance to either nutrient agar or nutrient broth. The medium was then neutralized and 0.1 per cent each of brom cresol purple (0.001 per cent alcoholic) and cresol red (0.001 per cent alcoholic) were added.

The ability to use purine bases, ammonia nitrogen, amino acid nitrogen, and urea was determined in neutralized media of the following composition with indicators added as before:

| | |
|---|---|
| Distilled water | 1,000 cc |
| Potassium chloride | 2 grams |
| Magnesium sulfate | 1 gram |
| D-glucose | 10 grams |
| Nitrogen-containing substance | 10 grams |

Growth and acid production were observed on each of the first five days and again on the tenth day. In some of the work nutrient broth was substituted for nutrient agar. The reduction of nitrates to nitrites was determined on nutrient agar to which 0.1 per cent of potassium nitrate had been added. Determinations of the presence of nitrites were made by adding a few drops of sulfanilic acid and α-naphthylamine solutions to the agar slants on the first, second, and fifth days after inoculation. Growth and the amount of gelatin liquefaction at 18° C were observed for a period of three months. Liquefaction within 5 days was recorded as rapid, 5 to 15 days as moderate, and over 15 days as slow. Failure to liquefy at the end of three months was regarded as indicating a non-liquefying culture. The gelatin tubes were sealed with paraffin to prevent drying out.

The growth in litmus milk was observed daily for ten days and with some unusual cultures which failed to alter the milk in ten days the period was extended to 20 days. The rapidity with which the litmus was reduced was assumed to be an indication of the rate of growth in those cultures which had the faculty of reducing litmus. Coagulation and peptonization was recorded as soon as noticed. The relation to oxygen requirements was observed as far as possible from the location of growth in Smith fermentation tubes.

## SELECTION OF CULTURES

The characters of the 197 cultures which survived the pasteurization treatment appear in Table 3. These data record the morphology, chromogenesis, Gram stain reactions, relation to oxygen, the power to liquefy gelatin and to reduce nitrates, the production of acid on d-glucose, lactose, and sucrose, the ability to use ammonia and amino acid nitrogen, and the reaction in litmus milk.

Table 4 gives the distribution of these cultures according to their morphology, chromogenesis, action on gelatin, and reduction of nitrates. The four largest groups were (1) the non-liquefying, non-nitrate-reducing white rods; (2) the liquefying, nitrate-reducing yellow cocci; (3) the liquefying, non-nitrate-reducing yellow cocci; and (4) the rods resembling *Streptococcus lactis*. Of these 197 cultures there

TABLE 3.—CHARACTERS OF THE 197 CULTURES FROM THE PASTEURIZED MILK PLATES WHICH SURVIVED PASTEURIZATION IN STERNBERG BULBS.

| Culture No. | History | | | Cultural characters | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Fermentation of | | | Milk reactions | | | | |
| | Date of receiving plates | Source* | Plate No. | Morphology† | Chromogenesis‡ | Gram stain | Aerobe | Gelatin liquefaction | Nitrate reduction | D-glucose | Lactose | Sucrose | Acid production | Acid production and coagulation | Peptonization | Utilization of ammonia nitrogen |
| 1 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | — | + | — | — | + |
| 2 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | + |
| 3 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | — | + | — | — | + |
| 4 | Nov. 6, '22 | K | 1 | R | w | + | + | — | — | + | + | + | + | — | — | + |
| 5 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | + |
| 6 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 7 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 8 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 9 | Nov. 6, '22 | K | 1 | R | w | + | + | — | — | + | + | + | + | — | — | ? |
| 9C | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | + |
| 10 | Nov. 6, '22 | K | 1 | R | w | + | + | — | — | + | + | + | + | — | — | ? |
| 11 | Nov. 6, '22 | K | 1 | R | w | + | + | — | — | + | + | + | + | — | — | ? |
| 12 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 13 | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 14C | Nov. 6, '22 | K | 1 | C | y | + | + | + | + | + | + | + | + | — | — | + |
| 15 | Nov. 16, '22 | J | 2 | R | w | — | + | — | + | — | — | — | — | — | + | ? |
| 16 | Nov. 16, '22 | J | 2 | R | w | — | + | — | + | — | — | — | — | — | + | ? |
| 17 | Nov. 16, '22 | J | 2 | R | w | + | + | — | — | + | + | + | + | — | — | ? |
| 18 | Nov. 16, '22 | J | 2 | C | w | + | + | — | — | + | + | + | + | + | — | — |
| 19 | Nov. 6, '22 | K | 3 | C | y | + | + | + | — | — | — | — | + | — | — | + |
| 20R | Nov. 6, '22 | K | 3 | R | w | + | + | — | — | + | + | + | + | — | — | + |
| 20Y | Nov. 6, '22 | K | 3 | C | y | + | + | + | — | — | — | — | + | — | — | + |
| 21 | Nov. 6, '22 | K | 3 | C | y | + | + | + | — | — | — | — | + | + | — | + |
| 22 | Nov. 6, '22 | K | 3 | C | y | + | + | — | + | + | + | — | + | — | — | + |
| 23 | Nov. 6, '22 | K | 3 | C | y | + | + | + | — | — | — | — | + | — | — | ? |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25 | Nov. 6, '22 | K | 3 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 26 | Nov. 6, '22 | K | 4 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 27 | Nov. 6, '22 | K | 4 | C | y | + | + | + | − | − | − | + | + | − | − | ? |
| 28 | Dec. 26, '22 | K | 5 | C | y | + | + | − | + | + | + | + | + | − | − | ? |
| 29 | Jan. 15, '23 | P | — | C | w | + | + | − | − | + | − | + | + | − | − | ? |
| 30 | Jan. 15, '23 | P | — | C | y | + | + | − | − | + | − | + | − | − | + | ? |
| 31 | Jan. 15, '23 | P | — | C | w | + | + | + | − | + | − | + | − | − | + | ? |
| 32 | Jan. 15, '23 | P | — | C | w | + | + | + | − | + | − | + | − | − | + | ? |
| 33 | Jan. 15, '23 | P | — | C | y | + | + | + | − | + | − | + | − | − | + | ? |
| 34 | Jan. 15, '23 | P | — | C | y | + | + | − | − | + | − | + | − | − | + | ? |
| 35 | Nov. 6, '22 | K | 3 | C | w | + | + | − | − | + | + | + | + | − | − | ? |
| 36 | Jan. 15, '23 | P | — | C | y | + | + | − | − | + | − | + | − | − | + | ? |
| 37 | Dec. 26, '22 | K | 6 | C | y | + | + | + | + | + | + | + | + | − | − | + |
| 38 | Dec. 26, '22 | K | 6 | C | y | + | + | + | + | + | + | + | + | − | − | ? |
| 39 | Dec. 26, '22 | K | 6 | C | y | + | + | − | + | + | + | + | + | − | − | − |
| 40 | Dec. 26, '22 | K | 6 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 41 | Jan. 26, '23 | C | 9 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 42 | Jan. 26, '23 | C | 9 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 43 | Jan. 26, '23 | C | 10 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 44 | Jan. 26, '23 | C | 10 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 45 | Jan. 26, '23 | C | 11 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 46 | Jan. 26, '23 | C | 11 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 47 | Jan. 26, '23 | C | 11 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 48 | Jan. 26, '23 | C | 11 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 49 | Jan. 26, '23 | C | 11 | C | w | + | + | − | − | + | + | + | + | − | − | ? |
| 50 | Jan. 26, '23 | C | 11 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 51 | Feb. 1, '23 | D | 12 | C | w | + | + | − | − | + | + | + | + | − | − | ? |
| 52 | Feb. 1, '23 | D | 12 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 53 | Feb. 1, '23 | D | 13 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 54 | Feb. 1, '23 | D | 13 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 55 | Jan. 19, '23 | D | 14 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 56 | Feb. 1, '23 | D | 16 | C | w | + | + | − | − | + | + | + | + | + | − | + |
| 57 | Feb. 1, '23 | D | 17 | C | w | + | + | + | + | + | + | + | + | + | − | − |
| 58 | Feb. 1, '23 | D | 18 | C | w | + | + | − | − | + | + | + | + | − | − | ? |
| 59 | Feb. 1, '23 | D | 18 | C | w | + | + | − | − | + | + | + | + | + | − | ? |

*Letters indicate as follows:
K—Kansas City, Mo.
J—Jackson, Mich.
P—Philadelphia, Pa.
C—Chicago, Ill.
D—Detroit, Mich.
B—Boston, Mass.
M—Milwaukee, Wis.
Ba—Baltimore, Md.

†C—coccus form; R—rod form.
‡w—white; y—yellow.

TABLE 3.—*Continued.*

| Culture No. | History | | | Cultural characters | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Date of receiving plates | Source* | Plate No. | Morphology† | Chromogenesis‡ | Gram stain | Aerobe | Gelatin liquefaction | Nitrate reduction | Fermentation of | | | Milk reactions | | | Utilization of ammonia nitrogen |
| | | | | | | | | | | D-glucose | Lactose | Sucrose | Acid production | Acid production and coagulation | Peptonization | |
| 60 | Feb. 1, '23 | D | 18 | C | w | + | + | — | — | + | + | + | + | — | — | ? |
| 61 | Feb. 1, '23 | D | 18 | C | w | + | + | — | — | + | + | + | + | — | — | ? |
| 62 | Feb. 1, '23 | D | 19 | C | w | + | + | — | — | + | + | + | + | — | — | ? |
| 63 | Dec. 26, '22 | K | 20 | C | w | + | + | — | — | + | + | + | + | — | — | ? |
| 64 | Feb. 16, '23 | B | 22 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 65 | Feb. 16, '23 | B | 22 | C | y | + | + | + | + | + | + | + | + | — | — | — |
| 66 | Feb. 16, '23 | B | 22 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 67 | Feb. 16, '23 | B | 22 | R | w | — | + | — | — | — | — | — | + | — | — | ? |
| 68 | Feb. 16, '23 | B | 22 | C | y | + | + | + | + | + | + | + | + | — | — | — |
| 69 | Feb. 16, '23 | B | 22 | C | w | + | + | + | — | + | + | + | + | + | — | ? |
| 70 | Feb. 16, '23 | B | 22 | C | y | + | + | + | + | + | + | + | + | — | — | + |
| 71 | Feb. 16, '23 | B | 22 | R | w | — | + | — | + | + | — | — | — | — | + | ? |
| 72 | Feb. 16, '23 | B | 22 | C | w | + | + | — | — | + | + | + | + | + | — | — |
| 73 | Feb. 16, '23 | B | 24 | C | w | + | + | — | + | + | + | — | + | — | — | ? |
| 74 | Jan. 23, '23 | M | 25 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 75 | Jan. 23, '23 | M | 25 | R | w | + | + | — | — | + | + | + | + | — | — | + |
| 76 | Jan. 23, '23 | M | 25 | R | w | + | + | + | — | + | + | + | + | — | — | ? |
| 77 | Jan. 23, '23 | M | 25 | C | w | + | + | + | + | + | + | + | + | + | — | ? |
| 78 | Jan. 23, '23 | M | 25 | C | w | + | + | + | + | + | + | + | + | + | — | ? |
| 79 | Jan. 23, '23 | M | 25 | R | y | + | + | + | — | + | + | + | + | + | — | ? |
| 80 | Feb. 14, '23 | K | 26 | C | y | + | + | — | + | + | + | + | + | — | — | ? |
| 81 | Feb. 14, '23 | K | 26 | C | y | + | + | — | + | + | + | + | + | + | — | ? |
| 82 | Feb. 14, '23 | K | 26 | R | w | + | + | — | — | + | + | + | + | — | — | ? |
| 83 | Feb. 14, '23 | K | 26 | C | w | + | + | — | — | + | + | + | + | + | — | ? |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 89 | Feb. 14, '23 | K | 27 | R | w | + | + | + | — | — | — | — | + | — | — | — | ? |
| 89A | Feb. 14, '23 | K | 27 | C | y | + | + | — | — | + | + | + | + | — | — | — | + |
| 90 | Feb. 14, '23 | K | 27 | R | y | + | + | + | — | — | — | — | + | — | — | — | ? |
| 90A | Feb. 14, '23 | K | 27 | C | y | + | + | + | + | + | + | + | + | — | — | — | + |
| 91 | Feb. 14, '23 | K | 27 | C | y | + | + | + | — | + | + | + | + | — | — | — | + |
| 92 | Feb. 14, '23 | K | 27 | C | w | + | + | + | + | + | + | — | + | — | — | — | + |
| 93 | Feb. 14, '23 | K | 27 | C | w | + | + | — | — | + | + | + | + | — | — | — | + |
| 93A | Feb. 14, '23 | K | 27 | R | y | + | + | — | — | + | + | + | + | — | — | — | ? |
| 94 | Feb. 14, '23 | K | 27 | R | w | + | + | + | — | — | — | — | + | — | — | — | ? |
| 94A | Feb. 14, '23 | K | 27 | C | y | + | + | — | — | + | + | + | + | — | — | — | + |
| 95 | Feb. 14, '23 | K | 27 | R | w | + | + | + | + | + | + | — | + | — | — | — | + |
| 95A | Feb. 14, '23 | K | 27 | C | y | + | + | — | — | + | + | + | + | — | — | — | + |
| 96 | Feb. 14, '23 | K | 27 | R | w | + | + | + | + | + | + | + | + | — | — | — | + |
| 96+ | Feb. 14, '23 | K | 27 | C | y | + | + | — | — | + | + | + | + | — | — | — | + |
| 97 | Feb. 14, '23 | K | 28 | R | w | + | + | — | + | + | + | — | + | — | — | — | ? |
| 97A | Feb. 14, '23 | K | 28 | C | w | + | + | — | — | + | + | + | + | — | — | — | + |
| 98 | Feb. 14, '23 | K | 28 | R | y | + | + | — | — | + | + | + | + | — | — | — | + |
| 98A | Feb. 14, '23 | K | 28 | R | y | + | + | — | + | + | + | — | + | — | — | — | + |
| 98B | Feb. 14, '23 | K | 28 | C | w | + | + | + | + | + | + | + | + | — | — | — | + |
| 98C | Feb. 14, '23 | K | 28 | C | y | + | + | — | — | + | + | + | + | — | — | — | + |
| 99 | Feb. 14, '23 | K | 28 | R | w | + | + | + | — | + | — | + | + | — | — | — | + |
| 99A | Feb. 14, '23 | K | 28 | C | y | + | + | — | — | + | + | + | + | — | — | — | + |
| 100 | Feb. 14, '23 | K | 28 | R | y | + | + | + | — | + | + | + | + | — | — | — | ? |
| 100A | Feb. 14, '23 | K | 28 | C | y | + | + | + | + | + | + | — | + | — | — | — | ? |
| 101 | Feb. 14, '23 | K | 28 | C | y | + | + | + | + | + | + | — | + | — | — | — | ? |
| 102 | Feb. 14, '23 | K | 28 | C | w | + | + | — | — | + | + | + | + | + | — | — | ? |
| 103 | Feb. 14, '23 | K | 28 | C | w | + | + | — | — | + | + | + | + | — | — | — | — |
| 104 | Feb. 14, '23 | K | 29 | R | y | + | + | — | — | + | + | + | + | — | — | — | ? |
| 105 | Feb. 14, '23 | K | 29 | R | y | + | + | + | + | + | + | + | + | + | — | — | ? |
| 106 | Feb. 14, '23 | K | 29 | C | w | + | + | + | + | + | + | + | + | — | — | — | ? |
| 107C | Feb. 14, '23 | K | 30 | C | y | + | + | — | — | + | + | + | + | — | — | — | + |
| 107R | Feb. 14, '23 | K | 30 | R | y | + | + | + | + | + | + | + | + | — | — | — | ? |
| 108 | Feb. 14, '23 | K | 30 | C | y | + | + | + | + | + | + | + | + | — | — | — | ? |
| 109 | Feb. 14, '23 | K | 30 | C | y | + | + | + | + | + | + | + | + | — | — | — | + |
| 110 | Feb. 14, '23 | K | 30 | C | y | + | + | + | + | + | + | + | + | + | — | — | + |
| 111 | Feb. 14, '23 | K | 30 | C | w | + | + | + | + | + | + | + | + | + | — | — | ? |
| 112 | Feb. 14, '23 | K | 31 | C | y | + | + | — | — | + | + | + | + | — | — | — | ? |
| 113 | Feb. 14, '23 | K | 31 | R | w | + | + | + | + | + | + | + | + | — | — | — | + |

*Letters indicate as follows:
K—Kansas City, Mo.
J—Jackson, Mich.
P—Philadelphia, Pa.
C—Chicago, Ill.
D—Detroit, Mich.
B—Boston, Mass.
M—Milwaukee, Wis.
Ba—Baltimore, Md.
†C—coccus form; R—rod form.
‡w—white; y—yellow.

TABLE 3.—*Concluded.*

| Culture No. | History | | | Cultural characters | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Date of receiving plates | Source* | Plate No. | Morphology† | Chromogenesis‡ | Gram stain | Aerobe | Gelatin liquefaction | Nitrate reduction | Fermentation of | | | Milk reactions | | | Utilization of ammonia nitrogen |
| | | | | | | | | | | D-glucose | Lactose | Sucrose | Acid production | Acid production and coagulation | Peptonization | |
| 114 | Feb. 21, '23 | D | 33 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 115 | Dec. 26, '22 | K | 32 | C | w | + | + | — | — | + | + | + | + | — | — | ? |
| 116 | Dec. 26, '22 | K | 32 | C | w | + | + | — | — | + | + | + | + | — | — | — |
| 117 | March 2, '23 | K | 35 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 118 | March 2, '23 | K | 35 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 119 | March 2, '23 | K | 35 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 120 | March 2, '23 | K | 35 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 121 | March 2, '23 | K | 36 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 122 | March 2, '23 | K | 36 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 123 | March 2, '23 | K | 36 | C | w | + | + | — | — | + | + | + | + | + | — | — |
| 124 | March 2, '23 | K | 36 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 125 | March 2, '23 | K | 37 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 126 | March 2, '23 | K | 37 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 127 | March 2, '23 | K | 37 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 128 | March 2, '23 | K | 37 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 129 | March 20, '23 | Ba | 38 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 130 | March 20, '23 | Ba | 39 | R | w | + | + | — | — | + | + | + | + | — | — | ? |
| 131 | March 20, '23 | Ba | 39 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 132 | March 20, '23 | Ba | 39 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 133 | March 20, '23 | Ba | 40 | C | w | + | + | + | + | + | + | + | + | — | — | ? |
| 134 | March 20, '23 | Ba | 40 | C | w | + | + | — | — | + | + | + | + | + | — | ? |
| 135 | March 20, '23 | Ba | 40 | C | w | + | + | — | — | + | + | + | + | — | — | ? |
| 136 | March 20, '23 | Ba | 42 | R | w | + | + | — | — | + | + | + | + | — | — | ? |
| 137C | March 20, '23 | Ba | 42 | C | y | + | + | + | + | + | + | + | + | — | — | ? |
| 138 | March 20, '23 | Ba | 42 | C | y | + | + | + | — | + | + | + | + | — | — | + |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 141Y | March 20, '23 | Ba | 42 | C | y | + | + | + | + | + | + | + | + | — | — | — | + |
| 141R | March 20, '23 | Ba | 42 | R | w | + | + | — | — | + | + | + | + | — | — | — | + |
| 142 | March 20, '23 | Ba | 42 | C | y | + | + | — | — | + | + | — | + | — | — | — | ? |
| 143 | March 20, '23 | Ba | 42 | C | w | + | + | — | — | + | — | — | + | — | — | — | ? |
| 144 | March 20, '23 | Ba | 42 | R | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 145 | March 20, '23 | Ba | 42 | C | y | + | + | — | + | + | + | — | + | — | — | — | ? |
| 146 | March 16, '23 | C | 43 | R | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 147 | March 16, '23 | C | 43 | C | w | + | + | — | — | + | + | — | + | — | — | — | ? |
| 148 | March 16, '23 | C | 43 | C | w | + | + | + | — | + | + | + | + | + | — | — | ? |
| 149 | March 16, '23 | C | 43 | C | w | + | + | + | — | + | + | + | + | + | — | — | ? |
| 150 | March 16, '23 | C | 43 | C | w | + | + | + | — | + | + | + | + | + | — | — | ? |
| 151 | March 16, '23 | C | 44 | C | w | + | + | + | — | + | + | + | + | + | — | — | ? |
| 152 | March 16, '23 | C | 44 | C | w | + | + | — | — | + | + | + | + | + | — | — | ? |
| 153 | March 16, '23 | C | 44 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 154 | March 16, '23 | C | 44 | C | w | + | + | — | — | + | + | + | + | + | — | — | ? |
| 155 | March 16, '23 | C | 45 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 156 | March 16, '23 | C | 45 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 157 | March 16, '23 | C | 45 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 158 | March 16, '23 | C | 45 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 159 | March 16, '23 | C | 46 | C | w | + | + | — | — | + | + | + | + | + | — | — | ? |
| 160 | March 16, '23 | C | 46 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 161 | March 16, '23 | C | 46 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 162 | March 16, '23 | C | 46 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 163 | March 16, '23 | C | 46 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 165 | March 16, '23 | C | 47 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 166 | March 16, '23 | C | 47 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 167 | March 16, '23 | C | 47 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 168 | March 16, '23 | C | 47 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 169 | March 16, '23 | C | 47 | C | w | + | + | — | — | + | + | + | + | + | — | — | ? |
| 170 | March 16, '23 | C | 47 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 171 | March 16, '23 | C | 47 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 172 | March 16, '23 | C | 48 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 173 | March 16, '23 | C | 48 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 174 | March 16, '23 | C | 48 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 175 | March 16, '23 | C | 48 | C | w | + | + | + | — | + | + | + | + | — | — | — | ? |
| 176 | March 16, '23 | C | 48 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 177 | March 16, '23 | C | 48 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 178 | March 16, '23 | C | 48 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 179 | March 16, '23 | C | 48 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |
| 180 | March 16, '23 | C | 48 | C | w | + | + | — | — | + | + | + | + | — | — | — | ? |

*Letters indicate as follows:
K—Kansas City, Mo.
J—Jackson, Mich.
P—Philadelphia, Pa.
C—Chicago, Ill.
D—Detroit, Mich.
B—Boston, Mass.
M—Milwaukee, Wis.
Ba—Baltimore, Md.
†C—coccus form; R—rod form.
‡w—white; y—yellow.

TABLE 4.—DISTRIBUTION OF THE 197 HEAT-RESISTANT CULTURES FROM PASTEURIZED MILK PLATES GROUPED ACCORDING TO MORPHOLOGY, CHROMOGENESIS, GELATIN LIQUEFACTION, AND NITRATE REDUCTION.

| MORPHOLOGY AND CHROMOGENESIS | GELATIN LIQUEFACTION | | | | TOTAL NUMBER OF CULTURES |
|---|---|---|---|---|---|
| | Positive | | Negative | | |
| | Nitrate reduction positive | Nitrate reduction negative | Nitrate reduction positive | Nitrate reduction negative | |
| Coccus, yellow..... | 38 | 15 | 8 | 5 | 66 |
| Coccus, white..... | 4 | 8 | 1 | 2 | 15 |
| Rods, yellow..... | 0 | 1 | 0 | 0 | 1 |
| Rod, white..... | 0 | 1 | 3 | 32 | 36 |
| Types resembling *Streptococcus lactis*. | 0 | 0 | 0 | 79 | 79 |

were 37 rods, 79 resembling *Streptococcus lactis*, and 81 cocci. Only 15 of these cocci were white, the remainder being yellow. Only one of the rods was yellow, the remainder being white. The white rods were difficult to grow on the agar slants, a fact which explains the failure to obtain more cultures of this type. Cultures of this type were relatively common on the pasteurized milk plates. A similar classification of Ayers and Johnson's (1913) cultures appears in Table 5.

## THERMAL RESISTANCE OF SPECIES STUDIED

The selection of the above heat-resistant cultures depended merely on the ability of an unknown number of organisms in 1 cc of milk to survive pasteurization in Sternberg bulbs. Representative cultures, therefore, were again selected from the series and more accurate data obtained to show the degree of resistance.

The literature is burdened with data based solely on a few tests on a milk culture, usually in open tubes, telling that this and that species is heat resistant. Such data do not indicate whether 5 or 95 per cent of the individual cells survive a given temperature. Gage and Stoughton (1906) report that the killing range is frequently as much as 35°C. The futility of such data appears more forcibly in Table 9. Only data where the thermal death point determinations or quantitative determinations of the numbers surviving are accurately made are of real value, and even these should be qualified with the conditions under which the tests were made.

Because of the bearing which this work has on pasteurization of milk, pure milk cultures, 15 hours old, have been pasteurized with this thought in mind. A DeKhotinsky electrically controlled water bath was provided to insure greater accuracy. The cultures were

TABLE 5.—DISTRIBUTION OF AYERS AND JOHNSON'S CULTURES FROM PASTEURIZED MILK ACCORDING TO MORPHOLOGY, CHROMOGENESIS, GELATIN LIQUEFACTION, AND NITRATE REDUCTION.*

| MORPHOLOGY | CHROMOGENESIS | GELATIN LIQUEFACTION | | | | | | TOTAL NUMBER OF CULTURES |
|---|---|---|---|---|---|---|---|---|
| | | Positive | | | Negative | | | |
| | | Nitrate reduction | | | Nitrate reduction | | | |
| | | Positive | Negative | Untested | Positive | Negative | Untested | |
| | | Acid-forming Group | | | | | | |
| Coccus | ± | 0 | 0 | 0 | 118 | 0 | 0 | 118 |
| Coccus | — | 0 | 0 | 9 | 0 | 0 | 0 | 9 |
| Rod | — | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Rod | — | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| | | Alkali-forming Group | | | | | | |
| Coccus | ± | 0 | 0 | 0 | 0 | 0 | 31 | 31 |
| Rod | ± | 0 | 0 | 0 | 0 | 0 | 12 | 12 |
| | | Peptonizing Group | | | | | | |
| Coccus | ± | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| ? | ± | 0 | 0 | 8 | 0 | 0 | 0 | 9 |
| ? | ± | 0 | 0 | 0 | 0 | 0 | 14 | 14 |
| ? | ± | 0 | 0 | 25 | 0 | 0 | 0 | 25 |
| | | Acid-forming Group | | | | | | |
| Coccus | — | 4 | 0 | 0 | 1 | 13 | 5 | 27 |
| Coccus | + | 7 | 9 | 5 | 0 | 0 | 0 | 21 |
| Rod | — | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Rod | yellow | 0 | 0 | 0 | 0 | 3 | 0 | 3 |
| | | Alkali-forming Group | | | | | | |
| Coccus | — | 0 | 0 | 0 | 1 | 2 | 0 | 3 |
| Coccus | yellow | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Rod | — | 0 | 0 | 0 | 1 | 1 | 0 | 2 |
| Rod | yellow | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| | | Peptonizing Group | | | | | | |
| Coccus | white | 3 | 2 | 0 | 1 | 2 | 0 | 8 |
| Coccus | yellow | 7 | 5 | 0 | 0 | 2 | 0 | 14 |
| Coccus | brown | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Rod | white | 6 | 4 | 0 | 1† | 1 | 0 | 12 |
| Rod | yellow | 1 | 0 | 0 | 0 | 1 | 0 | 2 |

*Adapted from *United States Dept. Agr. Bur. Animal Ind. Bul. No. 161.*
†Spore-forming rod.

TABLE 6.—PERCENTAGE INCREASE OR DECREASE IN PLATE COUNT AFTER HEATING AS INFLUENCED BY SUBJECTING MILK CULTURES OF SELECTED HEAT-RESISTANT BACTERIA TO 62.8°C FOR 30 MINUTES.

| CULTURE No. | TRIAL NUMBER | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | —38.4 | —86.0 | —53.7 | —41.7 | —42.9 | —35.7 | | |
| 2 | +23.9 | —92.0 | —36.5 | —30.0 | —25.0 | —27.6 | | |
| 3 | —99.9 | —99.9 | | | | | | |
| 4 | —77.7 | —99.0 | | | | | | |
| 5 | —99.8 | —80.0 | | | | | | |
| 6 | —99.3 | —98.4 | —98.6 | —98.8 | —98.9 | —89.0 | | |
| 7 | —99.0 | —73.0 | | | | | | |
| 9 | —27.7 | —95.0 | | | | | | |
| 9C | —32.7 | —24.0 | — 9.6 | —22.7 | | | | |
| 10 | —99.9 | —64.0 | —99.9 | —99.9 | —99.9 | —99.9 | | |
| 11 | + 7.2 | —92.0 | —99.9 | —99.9 | —99.9 | —99.9 | | |
| 12 | —99.6 | —86.8 | —88.9 | —89.7 | —83.5 | —87.0 | | |
| 13 | —95.0 | | | | | | | |
| 14C | —20.0 | —10.0 | —24.0 | —50.0 | | | | |
| 15 | —79.0 | —86.0 | —78.0 | —80.0 | —97.9 | —50.0 | | |
| 16 | —99.9 | —99.9 | —99.9 | —99.9 | —99.9 | —45.0 | | |
| 17 | +13.0 | + 6.5 | —26.0 | —23.4 | —26.5 | +10.1 | | |
| 18 | +23.6 | + 0.9 | — 2.7 | —15.0 | —15.1 | | | |
| 19 | +13.1 | +16.4 | + 7.0 | + 5.0 | —17.0 | —19.2 | —22.0 | —27.1 |
| 20R | +18.4 | + 5.1 | + 3.2 | — 1.4 | — 3.9 | — 9.5 | — 7.4 | —13.1 |
| 20Y | +11.4 | +21.2 | +13.1 | + 7.2 | + 5.1 | — 7.0 | —13.2 | —18.1 |
| 21 | —43.0 | —60.0 | —11.9 | —26.7 | —20.0 | — 9.4 | + 8.5 | + 9.0 |
| 22 | — 5.0 | —42.0 | —56.0 | —54.0 | —20.0 | —55.6 | | |
| 23 | —44.0 | —17.2 | — 2.3 | — 1.3 | — 3.7 | | | |
| 23R | +38.7 | +26.4 | +23.6 | — 9.3 | —00.0 | + 8.5 | — 2.3 | — 1.3 |
| 27 | —37.0 | —96.5 | —52.0 | —53.1 | —42.9 | —53.4 | | |
| 28 | —89.3 | —99.9 | —99.9 | —99.9 | —99.9 | | | |
| 29 | —23.0 | —93.9 | —92.4 | —96.8 | —86.9 | | | |
| 30 | —59.0 | —71.0 | +53.0 | —23.0 | —15.0 | — 61.0 | —37.8 | —33.7 |
| 31 | —61.0 | —99.9 | —99.9 | —99.9 | —99.9 | | | |
| 32 | —20.0 | +15.0 | — 7.0 | | | | | |
| 33 | —61.0 | —99.9 | —99.9 | —99.9 | —99.9 | | | |
| 34 | + 5.0 | —22.0 | —42.2 | —32.1 | +14.1 | —56.6 | —26.8 | —11.2 |
| 35 | +12.4 | — 1.1 | —40.0 | — 0.9 | —13.0 | —27.8 | —23.9 | + 4.7 |
| 36 | —37.0 | —17.0 | —15.0 | —25.0 | —60.0 | | | |
| 37 | —54.0 | —99.9 | —97.4 | —96.7 | —98.7 | | | |
| 38 | —99.9 | —99.9 | —99.9 | —99.9 | —99.9 | | | |
| 39 | —72.0 | —98.0 | —98.0 | —98.0 | —98.0 | | | |
| 56 | —99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 58 | —99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 66 | —99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 67 | —99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 68 | —99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 69 | —99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 70 | +70.0 | +25.9 | + 1.6 | —14.1 | —13.1 | —27.4 | —25.0 | —28.1 |
| 72 | +10.3 | +13.4 | +12.4 | — 0.0 | + 6.8 | + 9.7 | — 1.3 | +34.1 |
| 75 | +12.9 | +15.9 | —10.0 | — 4.8 | — 6.8 | — 9.9 | — 4.8 | —21.6 |
| 76 | —99.0 | | | | | | | |
| 77 | —50.0 | —52.0 | —58.0 | —60.0 | —11.6 | — 4.4 | —17.8 | — 6.8 |
| 78 | —95.0 | —95.0 | —96.0 | —95.0 | | | | |
| 80 | — 3.0 | —16.8 | — 6.3 | —12.3 | | | | |
| 85 | — 3.0 | — 0.3 | —12.3 | —16.0 | | | | |
| 86 | —99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 87 | +17.9 | — 4.9 | + 0.0 | — 5.0 | — 4.1 | —13.0 | — 9.8 | — 3.9 |
| 88 | —46.5 | —32.2 | —39.9 | —39.3 | | | | |
| 89 | —26.4 | —21.1 | + 3.4 | +14.0 | +11.1 | —24.7 | —22.2 | —36.4 |
| 90 | —44.9 | —49.8 | —30.0 | —32.9 | | | | |

TABLE 6.—*Continued.*

| CULTURE No. | TRIAL NUMBER | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 91 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 93 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 94 | + 2.1 | — 3.0 | —15.4 | —12.5 | —21.9 | —22.3 | —20.6 | —15.8 |
| 95 | — 99.9 | —99.9 | —99.9 | —99.9 | —47.0 | | | |
| 95A | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 96 | — 95.0 | —95.0 | —95.0 | —95.0 | | | | |
| 96+ | — 13.8 | + 0.5 | + 6.9 | + 7.9 | —18.9 | —13.1 | —17.3 | —20.3 |
| 97 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 98A | +120.0 | +91.9 | +83.3 | +18.2 | | | | |
| 98B | — 85.1 | —88.4 | —85.9 | —78.8 | | | | |
| 98C | + 13.1 | — 1.2 | + 7.4 | —10.7 | —14.2 | + 1.8 | | |
| 101 | — 46.9 | —22.6 | —28.2 | — 5.8 | | | | |
| 102R | + 16.0 | —18.0 | — 5.0 | +20.0 | —27.3 | —25.9 | —29.8 | |
| 102C | — 97.0 | —86.0 | —94.0 | —98.0 | —99.9 | —99.9 | —99.9 | —99.9 |
| 103 | + 13.5 | +16.3 | + 9.2 | + 6.1 | — 9.4 | — 5.3 | —21.7 | |
| 104 | — 9.2 | — 1.8 | — 7.9 | —23.7 | —18.3 | —92.0 | | |
| 105 | + 35.0 | +57.0 | | | | | | |
| 106 | — 11.0 | —18.0 | | | | | | |
| 107R | — 10.0 | —12.3 | —28.0 | —39.1 | | | | |
| 109 | — 74.5 | —68.7 | —66.5 | —51.9 | | | | |
| 110 | — 62.9 | —55.9 | —53.9 | —55.3 | | | | |
| 111 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 112 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 113 | — 6.8 | — 3.2 | +16.6 | +10.4 | + 1.4 | + 4.2 | | |
| 114 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 116 | — 89.6 | —88.7 | —90.5 | —73.8 | | | | |
| 117 | — 67.2 | —70.0 | —72.5 | —71.1 | | | | |
| 118 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 120 | — 23.6 | —47.6 | —33.3 | —63.3 | | | | |
| 123 | + 33.3 | +16.0 | +17.8 | +14.5 | —16.0 | | | |
| 124 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 129 | — 99.9 | —99.9 | —99.9 | —99.9 | —49.6 | —39.2 | —45.1 | —41.3 |
| 130 | — 4.4 | — 1.7 | — 2.1 | +10.7 | + 4.5 | +24.6 | — 5.8 | |
| 131 | — 9.0 | —32.0 | —18.4 | —26.2 | —31.6 | — 8.6 | | |
| 132C | + 1.5 | + 7.5 | — 2.7 | — 0.0 | —24.1 | —42.5 | —39.5 | |
| 136 | + 30.7 | +16.3 | + 6.4 | —13.8 | | | | |
| 137C | — 12.0 | —16.0 | — 6.0 | —61.0 | +30.0 | +10.0 | + 3.0 | —14.0 |
| 139 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 141R | + 29.8 | +10.4 | + 4.3 | + 6.3 | | | | |
| 141Y | + 25.1 | + 7.2 | — 8.2 | — 7.1 | —14.4 | —27.1 | —20.1 | |
| 142 | — 10.3 | —36.5 | —98.0 | —98.1 | —98.7 | —99.7 | | |
| 143 | — 98.0 | —98.0 | —98.0 | —98.0 | | | | |
| 144 | — 33.7 | —15.5 | — 8.5 | — 9.6 | | | | |
| 163 | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 16M | — 99.0 | —45.0 | | | | | | |
| 63M | — 84.2 | —59.9 | —92.4 | —83.3 | +31.0 | —38.0 | | |
| 412M | — 91.0 | —89.0 | —98.0 | —98.0 | —98.0 | —98.0 | | |
| 61M | — 99.9 | —99.9 | —99.9 | —99.9 | | | | |
| 1G | + 11.9 | — 0.4 | +39.4 | + 0.0 | — 7.8 | | | |
| 52G | + 31.3 | — 5.8 | + 4.6 | +14.4 | —11.4 | | | |
| 71G | + 19.0 | +13.7 | — 7.7 | —15.5 | +22.2 | | | |
| 76G | — 9.9 | +17.2 | + 7.1 | +44.2 | —18.8 | | | |
| 6B | + 51.8 | +21.0 | + 4.4 | —11.8 | — 7.7 | | | |
| 31B | + 20.1 | —12.5 | — 3.8 | +26.6 | +16.6 | | | |
| 41B | + 7.9 | — 1.4 | —14.5 | +17.1 | + 4.9 | | | |
| 48B | + 26.7 | +41.1 | +10.0 | — 3.0 | —19.8 | | | |
| 1Z | + 16.1 | + 9.0 | + 2.3 | — 7.1 | —13.3 | | | |
| 2Z | + 31.3 | — 7.7 | — 3.6 | +11.1 | — 3.9 | | | |
| H1 | + 7.1 | + 4.6 | — 1.4 | — 3.6 | — 5.3 | —18.3 | | |
| H19 | + 10.2 | + 6.1 | + 1.3 | — 7.6 | —10.0 | + 0.4 | | |

plated immediately before heating and immediately after cooling. Plates were incubated at 25°C for five days. From these data the percentage decrease or increase caused by heating was determined. These percentages are given in Table 6 with the number of trials on each of the selected cultures.

The common practice was to use a 0.1 or 0.01 dilution in milk, of the original 15-hour growth. The number of bacteria present even under these conditions was usually over a million per cc so that no data are submitted to show the influence of the dilution on the percentage of individuals killed. When a milk culture was sufficiently high in acid to coagulate upon the application of heat, the coagulum functioned as a protection for the cells against the penetration of heat. A probable explanation is the lack of mobility of the contents of the tube (Magoon and Culpepper, 1921).

In this way all cultures of which the majority thermal death point was less than 145°F for 30 minutes were eliminated. The final treatment left six cultures resembling *Streptococcus thermophilus*, six yellow sarcinae, two yellow micrococci, and nine cultures of a relatively small, dullish grey, non-motile, Gram-positive rod. In the case of several determinations of the percentage survival of the rods, a percentage increase ranging from 10 to 120 was obtained. Apparently, in some cases, the species had grown and in others the bacterial clumps had disintegrated during the heat treatment. Repeated experiments showed that no error had been made in diluting the samples or in marking the plates.

## EFFECT OF HEAT ON DISINTEGRATION OF BACTERIAL CLUMPS

The following cultures listed in Table 6 were chosen for further study because of their peculiar resistance. These cultures were grouped under four types according to the similarity of the characters displayed and in reality represent four species. Type I consisted of cultures Nos. 17, 20R, 75, 87, 98A, 113, 130, 136, and 141R; Type II of cultures Nos. 18, 23R, 35, 72, 103, and 123; Type III of cultures Nos. 19, 20Y, 70, 89, 96+, and 141Y; and Type IV of cultures Nos. 98C and 132. The sources of these cultures were as follows: Nos. 17 and 18 from Jackson, Mich.; Nos. 19, 20R, 20Y, 23R, 35, 87, 89, 96+, 98A, 98C, 103, 113, and 123 from Kansas City, Mo.; Nos. 70 and 72 from Boston; No. 75 from Milwaukee; and Nos. 130, 132, 136, 141R, and 141Y from Baltimore.

TABLE V.—INFLUENCE OF HEAT ON THE DISINTEGRATION OF THE BACTERIAL CLUMP IN MILK CULTURES.

| Type | Culture No. | Average size of clump in milk culture* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Unheated | Heated at 62.8°C for | | | | | | |
| | | | 10 minutes | 20 minutes | 30 minutes | | 60 minutes | 180 minutes | |
| | | | | | Size | Ratio $\frac{U}{H}$† | | Size | Ratio $\frac{U}{H}$† |
| I | 21 | 2.49 | | | 2.08 | 1.19 | | 2.04 | 1.22 |
| | 98A | 2.43 | | | 2.24 | 1.09 | | 2.09 | 1.16 |
| | 98A | 2.59 | | | 2.14 | 1.21 | | 2.09 | 1.24 |
| | 104 | 2.00 | | | 2.03 | 0.98 | | 1.88 | 1.07 |
| | 113 | 3.88 | | | 2.66 | 1.46 | | 1.85 | 2.10 |
| | 130 | 2.56 | | | 2.04 | 1.25 | | 1.88 | 1.36 |
| | 136 | 2.24 | | | 2.20 | 1.02 | | 2.15 | 1.04 |
| Average | | | | | | 1.17 | | | 1.31 |
| II | 23R | 2.65 | | | 2.28 | 1.17 | | 2.08 | 1.27 |
| | 35 | 2.92 | | | 2.15 | 1.36 | | 1.89 | 1.55 |
| | 72 | 2.62 | | | 2.28 | 1.15 | | 2.12 | 1.24 |
| | 103 | 2.00 | | | 1.85 | 1.07 | | 1.78 | 1.12 |
| | 123 | 1.84 | | | 1.65 | 1.12 | | 1.48 | 1.24 |
| Average | | | | | | 1.17 | | | 1.28 |
| III | 1Z | 36.7 | 28.8 | 32.4 | 28.4 | 1.29 | | 22.7 | 1.61 |
| | 1Z | 59.9 | 53.6 | 41.4 | 40.2 | 1.49 | 29.0 | 27.4 | 2.18 |
| | 2Z | 78.2 | 36.5 | 43.1 | 23.1 | 3.38 | 11.5 | 21.2 | 3.69 |
| | 2Z | 29.0 | | | 26.0 | 1.12 | | | |
| | 19 | 3.0 | | | 2.5 | 1.20 | | 2.0 | 1.50 |
| | 19 | 4.4 | | | 4.2 | 1.05 | | 3.7 | 1.16 |
| | 20 | 8.4 | | | 5.9 | 1.42 | | 6.5 | 1.30 |
| | 70 | 13.9 | | | 11.5 | 1.21 | | 10.4 | 1.34 |
| | 95 | 8.3 | | | 5.8 | 1.43 | | 5.4 | 1.54 |
| | 96+ | 8.1 | | | 6.5 | 1.25 | | 5.2 | 1.56 |
| Average | | | | | | 1.48 | | | 1.76 |

*Number of individual cells divided by the number of clumps observed gives the average number of cells per clump.

†Ratio $\frac{U}{H} = \frac{\text{Size of clump in unheated milk}}{\text{Size of clump after heating}}$.

The attempt to explain the results by assuming that the clump were broken up sufficiently to cause the entire percentage increase i unsatisfactory in the case of Types I and II. Nearly half as man more clumps appeared in the case of Type III at the end of heatin 30 minutes at 62.8°C as at the beginning. Table 7 shows the custom ary breaking up of the clumps of bacteria after the milk had bee subjected to pasteurization temperatures for different periods o time. On several occasions members of Type I lived for four an five days at 62.8°C.

### SURVIVAL AT PASTEURIZING TEMPERATURES

In order to determine how long species from these four types woul survive in milk cultures at pasteurizing temperatures, the followin technic was used. Cultures were first inoculated into milk an allowed to incubate at 25°C for 18 hours when they were placed a 62.8°C in a water bath. At different times, ranging between 15 an 240 minutes, a loopful of the milk culture was streaked on an aga slant which was then incubated at 25°C. At the end of seven day the amount of the growth was recorded. Table 8 demonstrate clearly that Types I and III survived better than Types II and IV Altho the technic employed was not above criticism, it indicate the relative ability of the different species to survive at the pasteu izing temperature.

### CULTURES FROM GENEVA, N. Y., AND BURLINGTON, VT., MILK SUPPLIE

The classification of the 132 cultures from the Geneva milk suppl is based on a knowledge of the following characters: (1) Morpholog as determined by the Gram stain of the agar slant culture and by th characteristic clumping of the bacteria in the milk culture; (2 chromogenesis on agar slants; (3) litmus milk reactions; and (4) ac production from d-glucose, lactose, and sucrose. Indicators we used to determine the approximate hydrogen-ion concentration the sugar broths after incubating the cultures for 10 days at 25°C The above determinations were run twice in duplicate within a perio of 12 months. The ability to ferment glycerol was determined durin the second series of tests. The same procedure was employed fo classifying the 41 cultures from the Burlington milk supply. Nearl all of the 35 coccus cultures from Geneva and the 7 from Burlingto were yellow sarcinae. No further tests were run on these cultures it was assumed that they were identical with the other members Type III.

TABLE 8.—SURVIVAL OF MILK CULTURES WHEN HEATED AT 62.8°C FOR VARYING PERIODS OF TIME.*

| PERIOD OF HEATING IN MINUTES | CULTURE NO., TYPE I | | | | | | | | CULTURE NO., TYPE II | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 17 | 20R | 75 | 87 | 98A | 98A | 113 | 130 | 18 | 23R | 35 | 72 | 103 | 132 |
| 15 | — | F | A | — | A | V | T | A | — | T | — | O | F | T |
| 20 | V | — | — | V | — | — | — | — | V | — | F | — | — | — |
| 30 | V | F | F | V | V | V | T | A | A | T | A | F | O | O |
| 60 | V | A | T | V | V | V | T | V | A | T | A | F | O | O |
| 90 | — | F | T | — | V | A | T | A | — | O | — | O | — | — |
| 120 | V | F | T | V | V | V | O | V | T | O | T | O | O | O |
| 180 | V | F | O | V | V | V | O | A | F | O | O | O | O | O |
| 240 | — | T | O | — | V | V | T | V | — | O | — | O | — | — |

| | CULTURE NO., TYPE III | | | | | | | | | CULTURE NO., TYPE IV | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 19 | 20Y | 70 | 89 | 96+ | 141Y | 1Z | 2Z | 95 | 98C | 132 |
| 15 | V | V | — | — | — | F | — | — | — | F | — |
| 20 | — | — | V | V | V | — | V | V | F | — | F |
| 30 | V | V | V | V | V | F | V | A | F | T | A |
| 60 | V | V | V | V | V | F | V | V | F | O | A |
| 90 | V | V | — | — | — | T | — | — | — | O | — |
| 120 | V | V | A | V | V | O | A | T | T | O | T |
| 180 | O | O | V | T | V | O | T | A | O | O | O |
| 240 | O | O | — | — | — | O | — | — | — | O | — |

*The symbols indicate as follows:
— =No test
O =No growth
T =Trace of growth
F =Fair growth
A =Abundant growth
V =Very abundant growth

The 97 rods from Geneva and the 34 from Burlington were identical and are considered as members of Type I. To present the data in detail is unnecessary. In Table 6 are included the percentages of increase or decrease in the plate counts of four typical cultures from each source when milk cultures of these species are subjected to pasteurizing temperatures.

### CULTURES FROM MISCELLANEOUS SOURCES

Similar data are included in Table 6 to show the percentage survival of cultures 1Z and 2Z (accidental isolations) and cultures H1 and H19 (isolations from plates sent to the Geneva laboratory by H. G. Harding).

### CULTURES FROM MILKING MACHINES

Milk cultures of the 415 cultures isolated from milking machines were subjected to pasteurization at different temperatures. Some of

TABLE 9.—RESULTS OF PASTEURIZING 159 OF THE 415 CULTURES FROM MILKING MACHINES.

| CULTURE No. | MILK CULTURES PASTEURIZED FOR 30 MINUTES IN | | | | | | | | | | | | APPROXIMATE INDIVIDUAL MICROSCOPIC COUNT IN MILLIONS PER CC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test tubes at | | | | | | | | Sternberg bulbs at | | | | |
| | 145°F | | 150°F | | 155°F | | 160°F | | 142°F | | 145°F | | |
| | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | |
| 1 | 6 | 3 | 3 | 1 | 4 | 1 | 4 | 2 | 1 | 0 | 2 | 0 | 20.0 |
| 4 | 6 | 2 | 3 | 0 | 2 | 1 | 2 | 0 | 1 | 0 | 2 | 0 | 3.0 |
| 5 | 7 | 4 | 4 | 2 | 2 | 2 | 4 | 4 | 1 | 0 | 2 | 0 | 7.0 |
| 16 | 5 | 1 | 3 | 0 | 2 | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 1.0 |
| 18 | 6 | 3 | 3 | 1 | 2 | 2 | 2 | 0 | 1 | 0 | 2 | 0 | 1.0 |
| 22 | 3 | 2 | 4 | 2 | 2 | 1 | 2 | 0 | 1 | 0 | 2 | 0 | 2.0 |
| 32 | 6 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 0 | 2 | 0 | 2.0 |
| 34 | 4 | 1 | 1 | 0 | – | – | – | – | 1 | 0 | 2 | 0 | 25.0 |
| 46 | 6 | 4 | 5 | 3 | 4 | 2 | 4 | 0 | 1 | 0 | 2 | 0 | 3.0 |
| 47 | 6 | 3 | 3 | 3 | 2 | 2 | 4 | 2 | 1 | 0 | 2 | 0 | 10.0 |
| 63 | 7 | 5 | 4 | 4 | 2 | 0 | 4 | 2 | 1 | 1 | 2 | 2 | 4.0 |
| 70 | 4 | 1 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 20.0 |
| 71 | 3 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 30.0 |
| 72 | 3 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 12.0 |
| 73 | 3 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 20.0 |
| 74 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 18.0 |
| 76 | 7 | 7 | 6 | 3 | 2 | 2 | 4 | 2 | 1 | 0 | 2 | 0 | 300.0 |
| 76 | – | – | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 2.0 |
| 77 | 5 | 1 | 3 | 3 | 2 | 2 | 4 | 2 | 1 | 0 | 2 | 0 | 1.0 |
| 78 | 2 | 0 | 1 | – | – | – | – | – | 1 | 0 | 2 | 0 | 15.0 |
| 79 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 16.0 |
| 81 | 6 | 3 | 4 | 3 | 2 | 0 | 4 | 2 | 1 | 0 | 2 | 0 | 1.0 |
| 83 | 5 | 2 | 3 | 1 | 2 | 1 | 2 | 0 | 1 | 1 | 2 | 2? | 65.0 |
| 84 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 10.0 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 85 | 5 | 3 | 3 | 2 | 2 | 2 | 4 | 0 | 1 | 0 | 2 | 0 | 2.0 |
| 86 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 30.0 |
| 87 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 5.0 |
| 88 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 45.0 |
| 89 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 2.0 |
| 90 | 5 | 2 | 3 | 1 | 2 | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 0.3 |
| 91 | 5 | 1 | 3 | 3 | 2 | 1 | 2 | 0 | 1 | 0 | 2 | 0 | 200.0 |
| 91 | – | – | – | – | – | – | – | – | 1 | 1? | 2 | 0 | 10.0 |
| 94 | 6 | 5 | 4 | 3 | 2 | 0 | 2 | 0 | 1 | 1 | 2 | 2 | 15.0 |
| 96 | 6 | 5 | 4 | 2 | 2 | 2 | 4 | 4 | 1 | 1 | 2 | 1 | 40.0 |
| 96 | – | – | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 8.0 |
| 98 | 2 | 0 | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 8.0 |
| 99 | 7 | 7 | 4 | 4 | 2 | 2 | 4 | 3 | 1 | 1 | 2 | 2 | 210.0 |
| 99 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 8.0 |
| 100 | 7 | 7 | 2 | 2 | 2 | 2 | 4 | 4 | 1 | 1 | 2 | 2 | 300.0 |
| 100 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 2.0 |
| 101 | 6 | 5 | 4 | 4 | 2 | 2 | 4 | 4 | 1 | 0 | 2 | 0 | 12.0 |
| 102 | 7 | 7 | 2 | 1 | 2 | 1 | 2 | 0 | 1 | 0 | 2 | 0 | 1.0 |
| 103 | 2 | 1 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 4.0 |
| 104 | 7 | 4 | 4 | 4 | 2 | 2 | 4 | 3 | 1 | 0 | 2 | 0 | 4.0 |
| 105 | 7 | 7 | 2 | 2 | 2 | 2 | 4 | 3 | 1 | 0 | 2 | 0 | 1.0 |
| 106 | 7 | 7 | 2 | 2 | 2 | 2 | 4 | 4 | 1 | 1 | 2 | 2 | 40.0 |
| 106 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 3.0 |
| 110 | 8 | 8 | 3 | 3 | 2 | 1 | 4 | 4 | 1 | 1 | 2 | 2 | 140.0 |
| 110 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 5.0 |
| 111 | 8 | 8 | 3 | 3 | 2 | 2 | 4 | 3 | 1 | 1 | 2 | 2 | 400.0 |
| 111 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 10.0 |
| 112 | 5 | 3 | 3 | 0 | 2 | 1 | 4 | 4 | 1 | 0 | 2 | 0 | 1.0 |
| 113 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 0.3 |
| 116 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 17.0 |
| 117 | 6 | 6 | 5 | 5 | 2 | 2 | 4 | 1 | 1 | 1 | 2 | 2 | 10.0 |
| 120 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 1.0 |
| 124 | 6 | 6 | 3 | 2 | 2 | 0 | 4 | 1 | 1 | 1 | 2 | 2 | 4.0 |
| 126 | 6 | 5 | 3 | 3 | 2 | 2 | 5 | 4 | 1 | 1 | 2 | 2 | 200.0 |
| 126 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 10.0 |

TABLE 9.—*Continued.*

| CULTURE No. | MILK CULTURES PASTEURIZED FOR 30 MINUTES IN | | | | | | | | | | | | APPROXIMATE INDIVIDUAL MICROSCOPIC COUNT IN MILLIONS PER CC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test tubes at | | | | | | | | Sternberg bulbs at | | | | |
| | 145°F | | 150°F | | 155°F | | 160°F | | 142°F | | 145°F | | |
| | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | |
| 127 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 3.0 |
| 128 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 10.0 |
| 129 | 8 | 6 | 5 | 4 | 4 | 4 | 4 | 3 | 1 | 1 | 2 | 2 | 25.0 |
| 130 | 8 | 3 | 6 | 1 | 4 | 0 | 4 | 1 | 1 | 0 | 2 | 0 | 10.0 |
| 137 | 4 | 2 | 4 | 2 | 2 | 1 | 4 | 4 | 1 | 0 | 2 | 0 | 1.0 |
| 141 | 6 | 4 | 3 | 2 | 2 | 1 | 4 | 1 | 1 | 0 | 2 | 0 | 4.0 |
| 142 | 4 | 1 | 2 | 0 | – | – | 2 | 0 | 1 | 0 | 2 | 0 | 7.0 |
| 142A | 6 | 6 | 3 | 3 | 1 | 0 | 2 | 1 | 1 | 1 | 2 | 2 | 400.0 |
| 142A | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 3.0 |
| 143 | 5 | 3 | 3 | 1 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 300.0 |
| 143 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 3.0 |
| 143A | 8 | 7 | 5 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 15.0 |
| 145A | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 3.0 |
| 147 | 5 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2.0 |
| 148 | 6 | 6 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 3.0 |
| 154A | 6 | 6 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 2 | 0 | 1.0 |
| 155 | 6 | 6 | 4 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 4.0 |
| 157 | 6 | 6 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2.0 |
| 158 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 5.0 |
| 158 | 6 | 6 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 300.0 |
| 159 | 6 | 6 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2.0 |
| 161 | 5 | 3 | 2 | 0 | – | – | – | – | 1 | 1 | 2 | 2 | 1.0 |
| 164 | 5 | 5 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 0 | 2 | 0 | 30.0 |
| 165 | 5 | 4 | 4 | 4 | 2 | 2 | 2 | 2 | 1 | 0 | 2 | 0 | 2.0 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 166 | 6 | 1 | 3 | 3 | 2 | 1 | 2 | 2 | 1 | 0 | 2 | 0 | 1.0 |
| 167 | 5 | 5 | 6 | 4 | 2 | 0 | 2 | 1 | 1 | 0 | 2 | 0 | 3.0 |
| 169 | 3 | 3 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 3.0 |
| 170 | 5 | 5 | 2 | 0 | 1 | 1 | 2 | 2 | 1 | 0 | 2 | 0 | 4.0 |
| 172 | 5 | 3 | 3 | 2 | 2 | 1 | 2 | 1 | 1 | 0 | 2 | 0 | 20.0 |
| 173 | 2 | 1 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 7.0 |
| 180 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 0 | 2 | 0 | 0.2 |
| 181 | 1 | 1 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 2 | 0 | 15.0 |
| 183 | 5 | 4 | 5 | 2 | 2 | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 10.0 |
| 185 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 40.0 |
| 190 | 4 | 2 | 2 | 0 | – | – | – | – | 1 | 0 | 2 | 0 | 4.0 |
| 192 | 2 | 0 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 20.0 |
| 196 | 2 | 2 | – | – | – | – | – | – | 1 | 0 | 2 | 0 | 30.0 |
| 205 | 6 | 6 | 4 | 4 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 100.0 |
| 206 | 4 | 4 | 2 | 2 | – | – | – | – | 1 | 0 | 2 | 0 | 1.0 |
| 215 | 6 | 5 | 4 | 4 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 1.0 |
| 226 | 6 | 6 | 4 | 3 | 2 | 2 | 2 | 0 | 1 | 1 | 2 | 2 | 5.0 |
| 228 | 6 | 3 | 4 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 2 | 0 | 17.0 |
| 229 | 3 | 3 | 2 | 1 | – | – | – | – | 1 | 0 | 2 | 0 | 2.0 |
| 234 | 6 | 4 | 4 | 4 | 2 | 1 | 2 | 0 | 1 | 0 | 2 | 0 | 10.0 |
| 266 | 4 | 2 | 4 | 1 | 2 | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 5.0 |
| 268 | 3 | 3 | 2 | 0 | 2 | 2 | 2 | 1 | 1 | 1 | 2 | 2 | 60.0 |
| 268 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 10.0 |
| 269 | 5 | 4 | 3 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 2 | 2 | 20.0 |
| 270 | 3 | 3 | 3 | 2 | 2 | 1 | 2 | 1 | 1 | 1 | 2 | 2 | 10.0 |
| 304 | 6 | 4 | 4 | 1 | 2 | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 20.0 |
| 331 | 3 | 3 | 3 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 2 | 2 | 20.0 |
| 331 | – | – | – | – | – | – | – | – | 1 | 1 | 2 | 2 | 30.0 |
| 352 | 6 | 6 | 4 | 4 | 2 | 2 | 2 | 2 | 1 | 0 | 2 | 0 | 4.0 |
| 353 | 1 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 1 | 0 | 2 | 0 | 2.0 |
| 359 | 4 | 2 | 2 | 0 | 1 | 0 | – | – | 1 | 0 | 2 | 0 | 1.0 |
| 369 | 6 | 6 | 4 | 2 | 2 | 0 | 2 | 1 | 1 | 1 | 2 | 2 | 25.0 |
| 372 | 3 | 1 | 3 | 1 | 2 | 0 | 2 | 0 | 1 | 1 | 2 | 2 | 3.0 |
| 374 | 4 | 2 | 2 | 1 | – | – | – | – | 1 | 0 | 2 | 0 | 20.0 |
| 375 | 6 | 5 | 4 | 3 | 2 | 1 | 2 | 0 | 1 | 0 | 2 | 0 | 4.0 |

TABLE 9.—*Concluded.*

| Culture No. | Milk cultures pasteurized for 30 minutes in | | | | | | | | | | | | Approximate individual microscopic count in millions per cc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test tubes at | | | | | | | | Sternberg bulbs at | | | | |
| | 145°F | | 150°F | | 155°F | | 160°F | | 142°F | | 145°F | | |
| | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | Number of trials | Number of survivals | |
| 378 | 4 | 3 | 1 | 1 | – | – | – | – | 1 | 0 | 2 | 0 | 4.0 |
| 379 | 3 | 1 | 3 | 2 | 2 | 0 | 2 | 1 | 1 | 0 | 2 | 0 | 2.0 |
| 390 | 3 | 2 | 3 | 1 | 2 | 1 | 2 | 0 | 1 | 0 | 2 | 0 | 3.0 |
| 409 | 6 | 5 | 4 | 3 | 2 | 2 | 2 | 0 | 1 | 0 | 2 | 0 | 3.0 |
| 411 | 4 | 3 | 2 | 1 | – | – | – | – | 1 | 1 | 2 | 2 | 10.0 |
| 412 | 3 | 2 | 2 | 1 | 2 | 1 | 2 | 0 | 1 | 1 | 2 | 2 | 10.0 |
| 417 | – | – | 2 | 1 | – | – | 2 | 0 | 1 | 0 | 2 | 0 | 2.0 |
| 418 | – | – | 2 | 1 | – | – | 2 | 0 | 1 | 0 | 2 | 0 | 2.0 |
| 427 | – | – | 2 | 0 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | 16.0 |
| 428 | – | – | 2 | 1 | – | – | 2 | 0 | 1 | 0 | 2 | 0 | 20.0 |
| 430 | – | – | 2 | 1 | – | – | 2 | 0 | 1 | 0 | 2 | 0 | —— |
| 431 | – | – | 2 | 0 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | —— |
| 434 | – | – | 2 | 1 | – | – | 2 | 0 | 1 | 0 | 2 | 0 | —— |
| 437 | – | – | 2 | 0 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | —— |
| 441 | – | – | 2 | 0 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | —— |
| 443Y | – | – | 2 | 0 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | —— |
| 444 | – | – | 2 | 2 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | —— |
| 448 | – | – | 2 | 1 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | —— |
| 451 | – | – | 2 | 1 | – | – | 2 | 1 | 1 | 1 | 2 | 2 | 3.0 |
| 452 | – | – | 2 | 2 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | —— |
| 453 | – | – | 2 | 0 | – | – | 2 | 2 | 1 | 0 | 2 | 0 | —— |
| 454 | – | – | 2 | 0 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | 6.0 |
| 455A | – | – | 2 | 1 | – | – | 2 | 0 | 1 | 0 | 2 | 0 | 30.0 |
| 469 | – | – | 2 | 0 | – | – | 2 | 1 | 1 | 0 | 2 | 0 | 15.0 |

| | | |

TABLE 10.—CHARACTERS OF THE 42 HEAT-RESISTANT MILKING MACHINE CULTURES WHICH SURVIVED PASTEURIZATION IN STERNBERG BULBS.*

| CULTURE No. | MORPHOLOGY | CHROMOGENESIS | GRAM STAIN | AEROBE | GELATIN LIQUEFACTION | NITRATE REDUCTION | FERMENTATION OF | | | MILK REACTIONS | | | USE OF AMMONIA NITROGEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | D-glucose | Lactose | Sucrose | Acid production | Acid production and coagulation | Peptonization | |
| 63 | C | y | + | + | + | − | − | − | − | + | − | − | − |
| 83 | C | w | − | + | + | + | + | + | + | − | − | − | ? |
| 94 | C | y | + | + | + | + | + | + | − | + | + | − | + |
| 96 | C | y | + | + | + | + | + | + | + | + | + | + | + |
| 98 | C | w | + | + | − | + | + | + | + | + | − | − | ? |
| 99 | R | y | + | + | + | + | + | + | − | + | + | + | ? |
| 100 | R | y | + | + | + | − | + | + | − | + | + | + | ? |
| 106 | C | y | + | + | + | − | + | + | + | + | + | + | ? |
| 110 | R | y | + | + | + | + | + | + | − | + | + | + | ? |
| 111 | R | y | + | + | + | − | + | + | − | + | + | + | ? |
| 117 | C | y | + | + | + | − | + | + | + | + | − | + | ? |
| 120 | C | w | + | + | + | − | + | + | − | + | + | − | − |
| 124 | C | y | + | + | + | + | + | + | − | + | + | − | + |
| 126 | C | y | + | + | − | − | + | − | − | + | + | − | − |
| 129 | C | w | + | + | − | − | + | + | + | + | + | − | ? |
| 142A | R | y | + | + | + | − | + | + | − | + | + | + | ? |

| | | |

the cultures were pasteurized in cotton-stoppered test tubes at 145°, 150°, 155°, and 160°F for 30 minutes and cooled to less than 40°F for 20 minutes. Agar slants were made from the milk tubes and incubated at 25°C for seven days. The amount and the distribution of the growth indicates in a limited manner the numbers of bacteria surviving the action of the heat. The milk cultures also were incubated for seven days when microscopic preparations were made to determine if growth had occurred. For comparison other similar data are submitted based on heating the same cultures in Sternberg bulbs at 142° and 145°C. After the desired heating and cooling the contents of the Sternberg bulbs were plated immediately. A partial quantitative determination of the percentage killed can be obtained in this manner. Only the results from heating 159 of the 415 cultures are presented in Table 9, as they are the only ones where the open tube treatment and Sternberg bulb method of heating could be compared. A greater number of the cells were killed in the Sternberg bulbs than when the same culture was heated in test tubes. Brown and Peiser (1916) argue that no advantage is obtained when the Sternberg bulb is used.

Only 42 cultures from milking machines among the 415 were able to survive pasteurization. A more complete description of these cultures appears in Table 10.

Studies to determine the percentage decrease or increase of the living bacteria in the milk cultures of the 42 cultures revealed nothing unusual. In all cases, except the yellow cocci and the three non-nitrate-reducing, non-liquefying rods, more than half of the cells were killed. At least twice as many yellow coccus cultures as cultures

TABLE 11.—DISTRIBUTION OF THE 42 HEAT-RESISTANT MILKING MACHINE CULTURES WHEN GROUPED ACCORDING TO MORPHOLOGY, CHROMOGENESIS, GELATIN LIQUEFACTION, AND NITRATE REDUCTION.

| MORPHOLOGY AND CHROMOGENESIS | GELATIN LIQUEFACTION | | | | TOTAL NUMBER OF CULTURES |
|---|---|---|---|---|---|
| | Positive | | Negative | | |
| | Positive nitrate reduction | Negative nitrate reduction | Positive nitrate reduction | Negative nitrate reduction | |
| Coccus, yellow..... | 10 | 5 | 1 | 2 | 18 |
| Coccus, white..... | 2 | 3 | 1 | 3 | 9 |
| Rod, yellow..... | 3 | 4 | 1 | 0 | 8 |
| Rod, white..... | 0 | 2 | 1 | 3 | 6 |
| Cultures resembling *Streptococcus lactis*. | 0 | 0 | 0 | 1 | 1 |

of any other one type survived. Further study showed that many of these were sarcinae. Table 11 contains a summary of the characters of the 42 cultures under discussion. All except 3 of the 42 heat-resistant species came from milking machines subjected to sterilization by chemicals, chiefly hypochlorites. Nearly two-thirds of these cultures were cocci. The data suggest that heat-resistant bacteria are more likely to be present in milk where chemical sterilization of milking machines is employed.

## DESCRIPTION OF TYPES ISOLATED

### QUALITATIVE STUDIES

The ability of 28 cultures to use certain of the carbohydrates, organic acids, amino acid nitrogen, and certain nitrogenous compounds has been determined. In a few cases where the cost of the test substances was prohibitive, the ability to use the substances was determined on only a few of the cultures from each type. These 28 cultures are distributed as follows: 10 in Type I, 6 in Type II, 8 in Type III, 2 in Type IV, and 2 in Type V. Because of the necessity of many repetitions, only a summary table (Table 12) indicating the final pH after ten days' incubation at 30°C is presented. No change in pH may indicate failure to grow or failure to liberate acid or alkali from the fermentable substance. An increase in alkalinity in broths containing organic acids occurs when the organic acid is utilized which liberates the alkali used to neutralize the broth. For the methods used, the reader is referred to the section under technic (page 14).

Type I may or may not ferment the pentoses and trisaccharides (raffinose); usually ferments the hexoses, disaccharides, glucosides, complex carbohydrates, and hexose and pentose alcohols; and generally fails to utilize the organic acids and amino acids. Hippuric acid is available.

Type II fails to ferment the pentoses and the amino acids; usually ferments the hexoses, disaccharides, trisaccharides, complex carbohydrates, and glucosides. Some of the hexose alcohols and glycerol are available, while the pentose alcohols are not. Some of the organic acids are utilized.

Type III ferments the pentoses, hexoses, disaccharides, trisaccharides, glucosides, and pentose and hexose alcohols. Some of the organic acids and amino acids are utilized.

TABLE 12.—UTILIZATION OF AND AVERAGE FINAL pH IN MEDIA CONTAINING VARIOUS FERMENTABLE SUBSTANCES AFTER GROWTH OF CULTURES FOR TEN DAYS AT 30°C.

| FERMENTABLE SUBSTANCE | TYPES | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | IV | | V | |
| | pH | Growth | pH | Growth | pH | Growth | pH | Growth | pH | Growth |
| Nutrient broth | 7.0 | + | 7.0 | + | 7.3 | + | 7.0 | + | 7.2 | + |
| Pentoses | | | | | | | | | | |
| Arabinose | 5.2 | ± | 7.0 | — | 8.1 | + | 5.0 | + | 7.6 | + |
| Xylose | 6.0 | ± | 7.0 | — | 7.4 | + | 5.7 | + | 7.5 | + |
| Hexoses | | | | | | | | | | |
| D-glucose | 4.9 | + | 5.0 | + | 7.6 | + | 5.3 | + | 7.5 | + |
| Fructose | 5.3 | + | 5.5 | + | 7.5 | + | 5.4 | + | 7.1 | + |
| Mannose | 5.1 | + | 5.1 | + | 7.4 | + | 5.6 | + | 7.7 | + |
| Galactose | 5.6 | + | 5.6 | + | 7.9 | + | 5.4 | + | 5.4 | + |
| Disaccharides | | | | | | | | | | |
| Lactose | 5.1 | + | 5.0 | + | 7.5 | + | 5.1 | + | 7.4 | + |
| Sucrose | 5.3 | + | 5.5 | + | 8.1 | + | 5.3 | + | 7.5 | + |
| Trisaccharide | | | | | | | | | | |
| Raffinose | 5.6 | ± | 5.3 | + | 8.3 | + | 5.9 | + | 7.4 | + |
| Glucosides | | | | | | | | | | |
| Amygdalin | 6.3 | + | 5.2 | + | 7.6 | + | 5.6 | + | 7.5 | + |
| Salicin | 5.2 | + | 4.7 | + | 7.9 | + | 5.1 | + | 7.0 | ? |
| Complex carbohydrates | | | | | | | | | | |
| Inulin | 7.2 | + | 7.4 | + | 8.3 | + | 5.8 | + | 7.4 | + |
| Dextrin | 6.6 | + | 6.1 | + | — | + | 6.0 | + | 6.7 | + |
| Soluble starch | 5.8 | + | 7.6 | + | 7.9 | + | 6.2 | + | 7.2 | + |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Alcohols | | | | | | | | | | |
| Mannite | 5.8 | + | 6.3 | + | 8.3 | + | 7.0 | ? | 6.8 | + |
| Dulcite | 6.2 | + | 7.0 | — | 7.6 | + | 6.5 | + | 6.7 | + |
| Sorbite | 6.6 | + | 7.2 | + | 7.8 | + | 7.6 | + | 7.7 | + |
| Adonite | 7.6 | ± | 7.0 | — | 7.6 | + | 7.4 | + | 7.6 | + |
| Erythrite | 6.4 | ± | 7.0 | — | 7.7 | + | 7.4 | + | 6.6 | + |
| Glycerol | 6.0 | + | 5.2 | + | 0.8 | + | 5.5 | + | 7.9 | + |
| Organic acids | | | | | | | | | | |
| Oxalic | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Malonic | — | ? | 8.0 | + | 7.5 | + | — | ? | — | ? |
| Succinic | 7.0 | — | 7.4 | + | 7.6 | + | 7.4 | + | 7.0 | — |
| Glycolic | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Lactic | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Malic | — | ? | 8.1 | + | 8.0 | + | 7.4 | + | 7.0 | — |
| Tartaric | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Citric | 7.0 | — | 7.0 | — | 7.9 | + | 7.0 | — | 7.0 | — |
| Oleic | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Fumaric | 7.0 | — | 7.4 | + | 7.0 | — | 7.0 | — | 7.0 | ? |
| Maleic | 7.0 | — | 7.0 | ? | 7.0 | — | 7.0 | — | 7.0 | — |
| Hippuric | 6.3 | + | 7.5 | + | 7.5 | + | 6.4 | + | 7.4 | + |
| Amino acids | | | | | | | | | | |
| Glycocoll | 7.0 | — | 7.0 | — | 5.6 | + | 7.0 | — | 7.0 | — |
| Glutamic acid | 7.0 | — | 7.0 | — | 5.8 | + | 7.0 | — | 7.2 | + |
| Aspartic acid | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.4 | + |
| Miscellaneous | | | | | | | | | | |
| Uric acid | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Nucleic acid | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Urea | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Lactose peptone bile | 7.0 | + | 7.0 | + | 7.0 | + | 7.0 | + | 7.0 | + |
| Sodium asparaginate | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |
| Ammonium citrate | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — | 7.0 | — |

Type IV ferments the pentoses, hexoses, disaccharides, trisac charides, glucosides, and pentose and hexose alcohols. Succinic malic, and hippuric acids are utilized. The amino acids are no available.

Type V ferments the pentoses, hexoses, disaccharides, trisaccha rides, glucosides, and pentose and hexose alcohols. Hippuric an some of the amino acids are fermented.

Uric acid, nucleic acid, urea, sodium asparaginate, and ammoniun citrate are not available as food for any of these types. Growt occurs in lactose peptone bile, altho there is no change in the pH

### QUANTITATIVE TESTS

Since the types of organisms were isolated from a product widel used for human consumption, further studies have been conducte to determine some of the quantitative changes in (1) the amount o sugars (d-glucose, lactose, and sucrose) used by representative specie from each of the five types. Nutrient broth containing 1 per cen of the fermentable substance was used. Determinations were als made of the lactose used when the cultures were growing in milk Calcium carbonate has been added to the solutions in some cases Studies on the production of (2) ammonia and (3) amino acid nitroge have been completed when the cultures were grown in the abov solutions. The amount of (4) the volatile acid produced in tw weeks and (5) the development of titratable acidity have been de termined. Alcohol and aldehyde tests were applied to the firs 10-cc portions of the volatile acid distillate. The results of the abov tests appear in Tables 13, 14, 15, 16, and 17.

### METHODS

The sugar determinations were made according to the Bertran (1906) method. The lactose in milk was determined by addin to 5 cc of milk in a 100-cc volumetric flask, 2 cc of a copper sulfat solution (34.639 grams of $CuSO_4 \cdot 5H_2O$ in 500 cc of distilled wate and 2 cc of a 0.5 N solution of sodium hydroxide. When the flask were half filled with water the contents were thoroly shaken. Th flasks were filled to the mark with water and the precipitated pro teins were filtered off. Lactose determinations were made of th filtrate according to the Bertrand (1906) method.

Ammonia nitrogen determinations were made by the Folin (1902 method. Sodium chloride was used in all determinations instea of sodium carbonate.

Amino acid nitrogen was determined according to the principles of the 'B method' of the original Sörensen (1908) test with partial modifications according to Brown (1923) and Levine and Carpenter (1923). Because of the small amount of amino acid nitrogen present, a 0.01N sodium hydroxide solution was employed for the titrations.

Volatile acid production was determined by the common steam distillation method from 1,000 cc of distillate. The presence or absence of alcohol in the first 10 cc of distillate was determined by the chromic acid method. The presence or absence of aldehyde and alcohol was determined by the iodoform test. All distillates gave negative results.

Type II attacks the carbohydrates actively. Type I is a little less active than Type II, while the remaining three Types attack them still more slowly. The nitrogenous compounds are more available to the species representing Types III and V than the carbohydrates in many instances. The titratable acidity increases with the amount of the carbohydrate utilized.

There was no appreciable change in the ammonia nitrogen content. Types I, II, and IV do not alter the amino acid nitrogen content. Type III shows a small increase and Type V shows just a slight increase.

The volatile acid production was detectable in Type IV. Type III produced a small amount, while Types I, II, and V failed to produce measurable amounts of volatile acids.

## SPECIES NAME ASSOCIATED WITH TYPES DESCRIBED

### TYPE I—*Microbacterium lacticum* ORLA-JENSEN, 1919

The size of this rod, together with its other characters, suggests the probable identity of this type with Orla-Jensen's *Microbacterium lacticum* (1919). The writer is not convinced, however, that Ayers and Johnson's *Lactobacillus thermophilus* (1924b) may not be the same species also.

Altho this species has been isolated from raw milk when the latter has been held at pasteurizing temperatures for several hours and from pasteurized milk plates, growth in sterilized skimmilk at pasteurizing temperatures when inoculated with laboratory strains has not been observed. The laboratory strains have been grown on agar slants for a period of more than a year, it being necessary to transfer them to new media about every four months. Before making

TABLE 13.—MILLIGRAMS OF SUGAR AT THE BEGINNING AND THE AMOUNTS USED UP AT THE END OF STATED PERIODS IN SOLUTIONS IN WHICH THE FIVE TYPES OF HEAT-RESISTANT BACTERIA ARE GROWING.

| TYPE No. | CULTURE No. | D-GLUCOSE BROTH | | | | | LACTOSE BROTH | | | | | SUCROSE BROTH | | | | | SKIMMILK | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | 3 da |
| I | 1G | 1,008 | 16 | 35 | 75 | 69 | 987 | 20 | 63 | 51 | 39 | 1,012 | 79 | 114 | 124 | 140 | 4,562 | 297 | 376 | 539 | 7 |
| | 1G | 842 | 43 | 49 | 59 | 80 | 1,083 | 18 | 33 | 23 | 62 | 985 | 67 | 81 | 104 | 93 | 4,649 | 288 | 346 | 600 | 5 |
| | 98A | 1,008 | 197 | 281 | 263 | 269 | 957 | 74 | 60 | 84 | 80 | 1,012 | 91 | 97 | 97 | 97 | 4,562 | 453 | 347 | 598 | 6 |
| | 98A | 842 | 82 | 100 | 118 | 118 | 987 | 49 | 68 | 94 | 82 | 985 | 43 | 49 | 67 | 77 | 4,649 | 384 | 404 | 493 | 5 |
| | 52G | —— | — | — | — | — | —— | — | — | — | — | —— | — | — | — | — | 4,159 | 231 | 308 | 433 | 4 |
| | 52G | —— | — | — | — | — | —— | — | — | — | — | —— | — | — | — | — | 4,159 | 105 | 231 | 243 | 3 |
| | 130 | —— | — | — | — | — | —— | — | — | — | — | —— | — | — | — | — | 4,159 | 58 | 172 | 413 | 3 |
| | 130 | —— | — | — | — | — | —— | — | — | — | — | —— | — | — | — | — | 4,159 | 152 | 192 | 279 | 4 |
| | 136 | 976 | 34 | 69 | 87 | 93 | 957 | 33 | 38 | 60 | 52 | 970 | 49 | 66 | 78 | 66 | 4,524 | 221 | 368 | 491 | 4 |
| | 136 | 842 | 20 | 40 | 40 | 46 | 987 | 30 | 58 | 90 | 99 | 985 | 25 | 40 | 49 | 67 | 4,562 | 134 | 297 | 453 | 3 |
| II | 23R | 1,008 | 126 | 197 | 222 | 222 | 1,083 | 86 | 154 | 164 | 190 | 1,012 | 91 | 137 | 162 | 194 | 4,649 | 493 | 540 | 695 | 8 |
| | 23R | 976 | 66 | 152 | 168 | 146 | 987 | 58 | 144 | 114 | 124 | 985 | 70 | 104 | 126 | 139 | 4,524 | 453 | 589 | 762 | 7 |
| | 103 | 1,008 | 72 | 92 | 157 | 178 | 987 | 68 | 58 | 94 | 114 | 1,012 | 52 | 94 | 120 | 159 | 4,562 | 211 | 396 | 509 | 5 |
| | 103 | 976 | 114 | 152 | 143 | 137 | 957 | 64 | 69 | 84 | 113 | 985 | 40 | 93 | 129 | 160 | 4,524 | 289 | 415 | 415 | 5 |
| III | 96+ | 1,008 | 13 | 29 | 53 | 66 | 957 | 28 | 33 | 24 | 33 | 1,012 | 30 | 42 | 42 | 49 | 4,649 | 444 | 636 | 948 | 9 |
| | 96+ | 842 | 25 | 43 | 28 | 56 | 987 | 20 | 58 | 54 | 68 | 970 | 25 | 49 | 34 | 28 | 4,524 | 259 | 406 | 667 | 7 |
| | 1Z | 1,008 | 16 | 44 | 101 | 86 | 1,083 | 9 | 53 | 67 | 145 | 1,012 | 52 | 76 | 91 | 86 | 4,524 | 163 | 368 | 279 | 3 |
| | 1Z | 976 | 21 | 66 | 65 | 75 | 957 | 33 | 38 | 52 | 60 | 970 | 52 | 62 | 82 | 62 | 4,562 | 125 | 288 | 406 | 4 |
| IV | 98C | 1,008 | 26 | 53 | 72 | 92 | 987 | 20 | 49 | 49 | 39 | 1,012 | 60 | 67 | 79 | 79 | 4,649 | 135 | 193 | 231 | 2 |
| | 98C | 842 | 15 | 34 | 49 | 34 | 957 | 33 | 38 | 64 | 84 | 970 | 44 | 34 | 55 | 82 | 4,524 | 135 | 211 | 192 | 2 |
| | 132 | 842 | 25 | 28 | 46 | 56 | 1,083 | 18 | 43 | 48 | 67 | 970 | 25 | 52 | 82 | 66 | 4,562 | 163 | 259 | 396 | 3 |
| | 132 | 976 | 34 | 60 | 63 | 60 | 987 | 34 | 68 | 49 | 86 | 985 | 33 | 12 | 59 | 70 | 4,524 | 173 | 221 | 279 | 3 |
| V | H1 | 1,008 | 13 | 29 | 44 | 50 | 1,083 | 33 | 126 | 96 | 86 | 1,012 | 46 | 86 | 171 | 140 | 4,524 | 96 | 163 | 299 | 2 |
| | H1 | 842 | 15 | 34 | 28 | 34 | 987 | 20 | 54 | 34 | 68 | 970 | 21 | 75 | 52 | 75 | 4,649 | 96 | 115 | 250 | 2 |
| | H19 | 842 | 18 | 25 | 46 | 34 | 957 | 28 | 64 | 80 | 84 | 970 | 52 | 66 | 55 | 89 | 4,649 | 39 | 77 | 154 | 2 |

TABLE 14.—CHANGES IN MILLIGRAMS PER 100 CC IN THE AMINO-ACID NITROGEN CONTENT (FORMOL TITRATION) OF SOLUTIONS IN WHI
THE FIVE TYPES OF HEAT-RESISTANT BACTERIA ARE GROWING.

| TYPE No. | CULTURE No. | D-GLUCOSE BROTH | | | | LACTOSE BROTH | | | | SUCROSE BROTH | | | | SKIMMILK | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 days | 7 days | 15 days | 30 days | 0 days | 7 days | 15 days | 30 days | 0 days | 7 days | 15 days | 30 days | 0 days | 7 days | 15 days | 30 |
| I | 1G | 13.41 | 15.76 | 13.66 | 14.22 | 13.41 | 21.84 | 14.11 | —— | 13.41 | 13.55 | 13.78 | 14.90 | 10.07 | 11.20 | 14.11 | 12 |
| | 98A | 13.41 | 12.99 | 12.77 | 13.55 | 13.41 | 14.90 | 15.34 | 14.00 | 13.41 | 19.26 | 16.80 | 22.06 | 10.07 | 9.63 | 12.77 | 15 |
| | 52G | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 10.07 | 14.56 | 13.22 | 11. |
| | 130 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 10.07 | —— | 12.54 | 19. |
| | 136 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 10.07 | 16.35 | 13.22 | 12. |
| II | 23R | 11.61 | 13.22 | 21.95 | 18.82 | 11.61 | 13.89 | 17.70 | 18.27 | 11.61 | 17.25 | —— | 19.82 | 10.07 | 15.68 | 13.22 | 10. |
| | 103 | 11.61 | 13.89 | 14.22 | 14.11 | 11.61 | 18.26 | 16.13 | 17.14 | 11.61 | 19.15 | 19.15 | 18.82 | 10.07 | 13.89 | 11.42 | 6. |
| III | 96+ | 11.61 | 20.16 | 18.51 | 12.10 | 11.61 | 13.66 | —— | 15.90 | 11.61 | 10.64 | 14.78 | 18.59 | 10.07 | 12.54 | 13.44 | 15. |
| | 1Z | 16.02 | 24.42 | 25.76 | 33.15 | 16.02 | 30.35 | 31.81 | 31.14 | 16.02 | 28.78 | 32.37 | 31.36 | 10.07 | 19.26 | 15.23 | — |
| IV | 98C | 11.41 | 14.22 | 15.79 | 16.46 | 11.41 | 14.90 | 17.47 | 16.35 | 11.41 | 16.80 | 14.56 | 17.14 | 10.07 | —— | 11.87 | 15. |
| | 132 | 13.41 | 17.58 | —— | 10.53 | 13.41 | 14.34 | —— | 14.45 | 13.41 | 13.66 | —— | 14.56 | 10.07 | 9.86 | 17.47 | 12. |
| V | H1 | 16.02 | 29.57 | 30.58 | 33.38 | 16.02 | 30.80 | 31.36 | 33.15 | 16.02 | 28.56 | 26.10 | 32.93 | 10.07 | 15.23 | 14.11 | 15. |
| | H19 | 13.41 | 12.43 | 15.46 | 16.02 | 13.41 | 16.35 | 16.24 | 15.90 | 13.41 | 18.59 | 20.50 | 18.93 | 10.07 | 14.56 | 13.66 | 17. |

TABLE 15.—CHANGES IN MILLIGRAMS PER 100 CC IN THE AMMONIA NITROGEN CONTENT OF SOLUTIONS IN WHICH THE FIVE TYPES OF HEAT-RESISTANT SPECIES ARE GROWING.

| Type No. | Culture No. | Plain broth | | D-glucose broth | | | | | Lactose broth | | | | | Sucrose broth | | | | | Skimmilk | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 days | 7 days | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | 30 day |
| I | 1G | 1.40 | 1.68 | 1.26 | 5.04 | 3.92 | 3.08 | 4.48 | 1.26 | 5.04 | 3.92 | 3.92 | 4.20 | 1.26 | 2.80 | 3.08 | 2.80 | 3.36 | 2.12 | 6.16 | 7.00 | 7.84 | 6.1 |
| | 1G | 1.26 | 2.52 | 1.26 | 4.48 | 4.48 | 3.92 | 3.64 | 1.26 | 5.04 | 4.48 | 4.48 | 4.76 | 1.26 | 3.64 | 4.20 | 3.08 | 2.80 | 2.12 | 7.84 | 6.44 | 5.88 | 7.0 |
| | 98A | 1.26 | 1.96 | 1.26 | 4.20 | 3.36 | 3.36 | 3.64 | 1.26 | 3.64 | 3.36 | 3.64 | 3.92 | 1.26 | 3.92 | 3.92 | 4.20 | 4.76 | 2.12 | 5.04 | 7.56 | 6.16 | 6.7 |
| | 98A | 1.26 | 1.96 | 1.26 | 4.76 | 4.20 | 3.92 | 4.48 | 1.26 | 4.20 | 3.92 | 4.48 | 4.48 | 1.26 | 4.48 | 3.64 | 4.48 | 4.20 | 2.12 | 5.60 | 5.88 | 7.28 | 5.6 |
| | 52G | 1.26 | 1.96 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 2.12 | 7.56 | 6.72 | 5.88 | 6.4 |
| | 52G | 1.26 | 2.24 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 2.12 | 5.32 | 6.16 | 7.00 | 6.4 |
| | 130 | 1.26 | 1.12 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 2.12 | 6.16 | 7.84 | 7.00 | 5.6 |
| | 130 | 1.26 | 1.40 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 2.12 | 5.60 | 6.72 | 6.44 | 6.7 |
| | 136 | 1.26 | 2.52 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 2.12 | 6.16 | 7.28 | 7.56 | 6.1 |
| | 136 | 1.26 | 1.68 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 2.12 | 7.28 | 5.60 | 8.12 | 7.2 |
| II | 23R | 1.26 | 3.08 | 1.26 | 4.20 | 3.92 | 5.32 | 3.92 | 1.26 | 5.88 | 3.92 | 5.04 | 6.44 | 1.26 | 3.08 | 3.64 | 4.20 | 3.92 | 2.12 | 3.08 | 3.64 | 3.64 | 4.7 |
| | 23R | 1.26 | 2.24 | 1.26 | 4.76 | 5.04 | 4.48 | 4.20 | 1.26 | 5.60 | 4.48 | 5.88 | 7.00 | 1.26 | 5.32 | 4.48 | 3.64 | 4.20 | 2.12 | 3.08 | 3.08 | 4.20 | 5.6 |
| | 103 | 1.26 | 1.12 | 1.26 | 5.18 | 4.48 | 5.04 | 4.48 | 1.26 | 5.04 | 5.88 | 4.48 | 4.76 | 1.26 | 3.92 | 4.76 | 4.76 | 5.60 | 2.12 | 3.36 | 3.92 | 3.64 | 4.7 |
| | 103 | 1.26 | 1.26 | 1.26 | 4.20 | 4.76 | 4.48 | 4.76 | 1.26 | 5.60 | 4.76 | 5.32 | 5.80 | 1.26 | 5.60 | 5.60 | 5.32 | 5.32 | 2.12 | 3.08 | 3.64 | 3.08 | 3.3 |
| III | 96+ | 1.26 | 3.92 | 1.26 | 5.04 | 2.80 | 3.36 | 2.80 | 1.26 | 4.76 | 3.36 | 4.20 | 4.20 | 1.26 | 3.08 | 3.08 | 3.36 | 3.36 | 2.12 | 7.28 | 8.96 | 11.20 | 10.0 |
| | 96+ | 1.26 | 2.80 | 1.26 | 3.64 | 3.36 | 4.76 | 3.92 | 1.26 | 5.04 | 3.92 | 4.76 | 5.04 | 1.26 | 2.52 | 3.64 | 3.92 | 3.64 | 2.12 | 8.96 | 11.20 | 11.20 | 11.2 |
| | 1Z | 1.26 | 4.48 | 1.26 | 3.64 | 2.52 | 3.36 | 3.64 | 1.26 | 4.76 | 2.80 | 2.80 | 5.04 | 1.26 | 4.48 | 4.76 | 3.92 | 3.76 | 2.12 | 5.32 | 7.28 | 9.52 | 9.5 |
| | 1Z | 1.26 | 5.32 | 1.26 | 4.48 | 3.64 | 4.48 | 4.76 | 1.26 | 4.76 | 4.20 | 3.36 | 4.48 | 1.26 | 3.92 | 3.64 | 3.36 | 4.20 | 2.12 | 7.56 | 8.12 | 8.96 | 10.3 |
| IV | 98C | 1.26 | 3.36 | 1.26 | 3.92 | 1.96 | 4.48 | 3.92 | 1.26 | 3.92 | 3.36 | 4.20 | 3.36 | 1.26 | 4.76 | 3.92 | 4.20 | 3.64 | 2.12 | 8.40 | 8.68 | 8.68 | 9.2 |
| | 98C | 1.26 | 2.52 | 1.26 | 4.20 | 2.24 | 5.04 | 4.76 | 1.26 | 3.92 | 3.92 | 3.08 | 4.76 | 1.26 | 4.20 | 3.64 | 3.08 | 3.08 | 2.12 | 6.72 | 8.40 | 9.52 | 10.3 |
| | 132 | 1.26 | 4.48 | 1.26 | 2.80 | 3.08 | 2.80 | 3.92 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 2.12 | 5.32 | 6.16 | 7.84 | 8.4 |
| | 132 | 1.40 | 3.36 | 1.26 | 2.24 | 4.76 | 3.36 | 3.08 | —— | —— | —— | —— | —— | —— | —— | —— | —— | —— | 2.12 | 6.44 | 7.84 | 8.96 | 8.4 |
| V | H1 | 1.40 | 5.04 | 1.26 | 4.20 | 4.48 | 5.60 | 4.48 | 1.26 | 5.32 | 5.04 | 6.44 | 4.76 | 1.26 | 4.76 | 5.04 | 5.04 | 5.32 | 2.12 | 4.48 | 5.60 | 3.92 | 4.4 |
| | H1 | 1.40 | 3.92 | 1.26 | 3.08 | 3.92 | 5.60 | 4.76 | 1.26 | 4.48 | 5.60 | 5.60 | 3.36 | 1.26 | 4.20 | 4.48 | 5.60 | 5.60 | 2.12 | 6.72 | 5.32 | 5.32 | 5.0 |
| | H19 | 1.26 | 3.64 | 1.26 | 2.80 | 3.64 | 4.48 | 4.48 | 1.26 | 3.08 | 4.48 | 4.48 | 4.76 | 1.26 | 5.32 | 4.20 | 4.48 | 5.04 | 2.12 | 5.04 | 6.16 | 5.60 | 4.7 |
| | H19 | 1.26 | 4.20 | 1.26 | 3.64 | 3.08 | 5.04 | 4.76 | 1.26 | 4.48 | 3.64 | 4.20 | 3.64 | 1.26 | 4.76 | 5.04 | 5.04 | 4.76 | 2.12 | 5.88 | 5.60 | 4.76 | 5.8 |

TABLE 16.—CHANGES IN GRAMS PER 100 CC IN THE TITRATABLE ACIDITY OF SOLUTIONS IN WHICH THE FIVE SPECIES OF HEAT-RESISTANT ORGANISMS ARE GROWING.

| TYPE NO. | CULTURE NO. | D-GLUCOSE BROTH | | | | | LACTOSE BROTH | | | | | SUCROSE BROTH | | | | | SKIMMILK | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | 30 days | 0 days | 3 days | 7 days | 15 days | |
| I | 1G | 0.030 | 0.050 | 0.070 | 0.095 | 0.105 | 0.030 | 0.070 | 0.075 | 0.100 | 0.130 | 0.030 | 0.055 | 0.065 | 0.070 | 0.090 | 0.165 | 0.280 | 0.325 | 0.330 | 0 |
| | 1G | 0.030 | 0.060 | 0.085 | 0.120 | 0.090 | 0.030 | 0.045 | 0.060 | 0.105 | 0.150 | 0.030 | 0.045 | 0.055 | 0.035 | 0.065 | 0.165 | 0.210 | 0.240 | 0.280 | 0 |
| | 98A | 0.030 | 0.080 | 0.095 | 0.105 | 0.110 | 0.030 | 0.100 | 0.105 | 0.130 | 0.145 | 0.030 | 0.070 | 0.095 | 0.110 | 0.125 | 0.165 | 0.220 | 0.270 | 0.330 | 0 |
| | 98A | 0.030 | 0.095 | 0.080 | 0.090 | 0.100 | 0.030 | 0.110 | 0.115 | 0.130 | 0.160 | 0.030 | 0.100 | 0.095 | 0.115 | 0.115 | 0.165 | 0.260 | 0.280 | 0.315 | 0 |
| | 52G | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 0.165 | 0.270 | 0.295 | 0.310 | 0 |
| | 52G | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 0.165 | 0.250 | 0.270 | 0.300 | 0 |
| | 130 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 0.165 | 0.300 | 0.310 | 0.325 | 0 |
| | 130 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 0.165 | 0.240 | 0.275 | 0.300 | 0 |
| | 136 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 0.165 | 0.295 | 0.310 | 0.325 | 0 |
| | 136 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | 0.165 | 0.325 | 0.350 | 0.380 | 0 |
| II | 23R | 0.030 | 0.115 | 0.160 | 0.205 | 0.220 | 0.030 | 0.190 | 0.185 | 0.160 | 0.170 | 0.030 | 0.220 | 0.240 | 0.270 | 0.275 | 0.165 | 0.610 | 0.660 | 0.680 | 0 |
| | 23R | 0.030 | 0.150 | 0.200 | 0.230 | 0.260 | 0.030 | 0.175 | 0.160 | 0.125 | 0.170 | 0.030 | 0.190 | 0.200 | 0.275 | 0.250 | 0.165 | 0.720 | 0.740 | 0.780 | 0 |
| | 103 | 0.303 | 0.140 | 0.175 | 0.190 | 0.230 | 0.030 | 0.160 | 0.165 | 0.170 | 0.180 | 0.030 | 0.200 | 0.215 | 0.240 | 0.285 | 0.165 | 0.700 | 0.720 | 0.740 | 0 |
| | 103 | 0.030 | 0.160 | 0.225 | 0.235 | 0.250 | 0.030 | 0.210 | 0.180 | 0.190 | 0.200 | 0.030 | 0.225 | 0.215 | 0.255 | 0.270 | 0.165 | 0.680 | 0.710 | 0.735 | 0 |
| III | 96+ | 0.030 | 0.090 | 0.110 | 0.130 | 0.170 | 0.030 | 0.055 | 0.075 | 0.090 | 0.110 | 0.030 | 0.045 | 0.055 | 0.070 | 0.110 | 0.165 | 0.400 | 0.460 | 0.495 | 0 |
| | 96+ | 0.030 | 0.085 | 0.085 | 0.145 | 0.160 | 0.030 | 0.065 | 0.100 | 0.110 | 0.135 | 0.030 | 0.065 | 0.070 | 0.125 | 0.145 | 0.165 | 0.420 | 0.580 | 0.560 | 0 |
| | 1Z | 0.030 | 0.120 | 0.150 | 0.160 | 0.185 | 0.030 | 0.110 | 0.145 | 0.140 | 0.170 | 0.030 | 0.065 | 0.070 | 0.090 | 0.110 | 0.165 | 0.340 | 0.390 | 0.440 | 0 |
| | 1Z | 0.030 | 0.145 | 0.180 | 0.200 | 0.230 | 0.030 | 0.130 | 0.155 | 0.160 | 0.185 | 0.030 | 0.110 | 0.155 | 0.170 | 0.175 | 0.165 | 0.425 | 0.440 | 0.495 | 0 |
| IV | 98C | 0.030 | 0.125 | 0.120 | 0.140 | 0.160 | 0.030 | 0.090 | 0.100 | 0.120 | 0.135 | 0.030 | 0.080 | 0.120 | 0.125 | 0.140 | 0.165 | 0.410 | 0.425 | 0.450 | 0 |
| | 98C | 0.030 | 0.080 | 0.150 | 0.090 | 0.105 | 0.030 | 0.095 | 0.125 | 0.120 | 0.145 | 0.030 | 0.045 | 0.085 | 0.110 | 0.125 | 0.165 | 0.400 | 0.410 | 0.450 | 0 |
| | 132 | 0.030 | 0.090 | 0.115 | 0.140 | 0.170 | — | — | — | — | — | — | — | — | — | — | 0.165 | 0.400 | 0.420 | 0.455 | 0 |
| | 132 | 0.030 | 0.130 | 0.130 | 0.165 | 0.170 | — | — | — | — | — | — | — | — | — | — | 0.165 | 0.440 | 0.435 | 0.445 | 0 |
| V | H1 | 0.030 | 0.100 | 0.125 | 0.140 | 0.155 | 0.030 | 0.090 | 0.100 | 0.100 | 0.110 | 0.030 | 0.080 | 0.090 | 0.115 | 0.130 | 0.165 | 0.300 | 0.310 | 0.335 | 0 |
| | H1 | 0.030 | 0.125 | 0.140 | 0.160 | 0.175 | 0.030 | 0.110 | 0.145 | 0.165 | 0.165 | 0.030 | 0.100 | 0.120 | 0.155 | 0.155 | 0.165 | 0.260 | 0.275 | 0.300 | 0 |
| | H19 | 0.030 | 0.090 | 0.100 | 0.110 | 0.115 | 0.030 | 0.085 | 0.090 | 0.110 | 0.125 | 0.030 | 0.075 | 0.095 | 0.110 | 0.130 | 0.165 | 0.250 | 0.270 | 0.295 | 0 |
| | H19 | 0.030 | 0.085 | 0.090 | 0.095 | 0.101 | 0.030 | 0.080 | 0.085 | 0.120 | 0.140 | 0.030 | 0.100 | 0.105 | 0.110 | 0.125 | 0.165 | 0.325 | 0.325 | 0.325 | 0 |

TABLE 17.—VOLATILE ACID CONTENT OF SOLUTIONS IN WHICH THE FIVE SPECIES OF HEAT-RESISTANT ORGANISMS ARE GROWING.
(Number of cc of N/10 NaOH to neutralize volatile acid in 100 cc of solution.)

| Type No. | Culture No. | Plain broth | | | D-glucose broth | | | Lactose broth | | | Sucrose broth | | | Skimmilk | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Uninoculated | Culture 10 to 14 days old | | Uninoculated | Culture 10 to 14 days old | | Uninoculated | Culture 10 to 14 days old | | Uninoculated | Culture 10 to 14 days old | | Uninoculated | Culture 10 to 14 days old | |
| | | | Without $CaCO_3$ | With $CaCO_3$ | | Without $CaCO_3$ | With $CaCO_3$ | | Without $CaCO_3$ | With $CaCO_3$ | | Without $CaCO_3$ | With $CaCO_3$ | | Without $CaCO_3$ | With $CaCO_3$ |
| I | 1G | 1.6 | 1.9 | 1.8 | 1.6 | 2.4 | 2.5 | 1.6 | — | 3.6 | 1.6 | 1.4 | 3.3 | 2.0 | 2.5 | — |
| | 1G | 1.6 | 1.7 | 1.8 | 1.6 | 2.9 | .21 | 1.6 | — | 3.1 | 1.6 | 1.4 | 3.6 | 2.0 | 3.3 | — |
| | 98A | 1.6 | 1.8 | 1.7 | 1.6 | 2.8 | — | 1.6 | 2.5 | — | 1.6 | 2.4 | — | 2.0 | 5.3 | 6.3 |
| | 98A | 1.6 | 1.4 | 1.8 | 1.6 | 2.4 | — | 1.6 | 2.3 | — | 1.6 | 2.5 | — | 2.0 | 4.5 | 4.8 |
| | 52G | 1.6 | 1.6 | — | 1.6 | 3.2 | — | 1.6 | 3.7 | — | 1.6 | 1.6 | — | — | — | — |
| | 52G | 1.6 | 1.7 | — | 1.6 | 2.6 | — | 1.6 | 2.5 | — | 1.6 | 1.8 | — | — | — | — |
| | 130 | 1.6 | 1.0 | — | — | — | — | — | — | — | — | — | — | 2.0 | 4.2 | — |
| | 130 | 1.6 | 1.2 | — | — | — | — | — | — | — | — | — | — | 2.0 | 3.0 | — |
| | 136 | 1.6 | 1.7 | 1.8 | 1.6 | 2.5 | — | — | — | — | — | — | — | 2.0 | 3.7 | — |
| | 136 | 1.6 | 1.6 | 1.8 | 1.6 | 2.4 | — | — | — | — | — | — | — | 2.0 | 3.2 | — |
| II | 23R | 1.6 | 1.9 | 1.8 | 1.6 | 2.4 | 2.5 | 1.6 | 3.3 | 3.0 | 1.6 | 2.1 | 3.6 | 2.0 | 3.6 | 4.8 |
| | 23R | 1.7 | 1.9 | 1.6 | 1.6 | 2.0 | 2.6 | 1.6 | 1.9 | 2.2 | 1.6 | 2.1 | 2.9 | 2.0 | 4.5 | 5.1 |
| | 103 | 1.6 | 2.1 | 2.2 | 1.6 | 1.7 | — | 1.6 | 1.6 | — | 1.6 | 1.6 | — | 2.0 | 3.4 | 4.2 |
| | 103 | 1.6 | 2.3 | 2.0 | 1.6 | 2.1 | — | 1.6 | 1.9 | — | 1.6 | 1.7 | — | 2.0 | 3.9 | 4.1 |
| III | 96+ | 1.6 | 2.4 | 2.8 | 1.6 | 2.4 | — | 1.6 | 2.0 | — | 1.6 | 2.3 | — | 2.0 | 8.6 | — |
| | 96+ | 1.6 | 2.7 | 2.4 | 1.6 | 2.2 | — | 1.6 | 2.2 | — | 1.6 | 1.7 | — | 2.0 | 7.3 | — |
| | 1Z | 1.6 | 2.0 | 2.6 | 1.6 | 1.5 | 3.9 | 1.6 | 1.9 | 3.7 | 1.6 | 1.9 | 3.9 | 2.0 | 5.0 | — |
| | 1Z | 1.6 | 2.2 | 2.4 | 1.6 | 1.9 | 3.4 | 1.6 | 2.0 | 3.8 | 1.6 | 2.0 | 4.1 | 2.0 | 4.6 | — |
| IV | 98C | 1.7 | 3.1 | 3.0 | 1.6 | 4.8 | 13.2 | 1.6 | 4.5 | 17.3 | 1.6 | 4.2 | 13.3 | 2.0 | 17.0 | 19.2 |
| | 98C | 1.6 | 2.6 | 2.8 | 1.6 | 4.5 | 12.0 | 1.6 | 4.0 | 13.4 | 1.6 | 4.1 | 12.5 | 2.0 | 14.0 | 16.7 |
| | 132 | 1.6 | 3.4 | 3.0 | 1.6 | 7.0 | — | 1.6 | 5.5 | — | 1.6 | 6.1 | — | 3.0 | 21.0 | — |
| | 132 | 1.6 | 3.6 | 2.6 | 1.6 | 8.4 | — | 1.6 | 5.4 | — | 1.6 | 4.9 | — | 2.0 | 18.0 | — |
| | H1 | 1.6 | 1.4 | 1.8 | 1.6 | 1.4 | 2.4 | 1.6 | 1.6 | 1.5 | 1.6 | 1.6 | 1.5 | 3.0 | 4.1 | — |
| | H1 | 1.6 | 1.5 | 1.8 | 1.6 | 1.6 | 2.8 | 1.6 | 1.5 | 2.0 | 1.6 | 1.7 | 2.0 | 2.0 | 4.2 | — |

the tests, the cultures were grown in sterile litmus milk for several generations in order to increase their vitality.

Whether freshly isolated cultures would grow at pasteurizing temperatures in raw milk has not been determined. Ayers and Johnson's rod is reported to be larger than the form which appears in the observed laboratory strains.

### TYPE II—*Streptococcus thermophilus* ORLA-JENSEN, 1919

Orla-Jensen (1919) found that the commonest lactic acid bacterium, *Streptococcus lactis* (Lister) Löhnis, was killed at 70°C in 15 minutes. This species usually coagulates milk in less than 24 hours at temperatures ranging between 25° and 37°C. *Streptococcus mastiditis* Migula is killed at 60°C in 15 minutes (Orla-Jensen, 1919). Ayers and Johnson (1918) found that 27 strains of pathogenic streptococci were killed in 30 minutes at 140°F when heated in milk. It is evident that Type II is more resistant to heat than the above-mentioned species.

Orla-Jensen described a *Streptococcus thermophilus* which survived in large numbers a temperature of 70°C for 15 minutes but was nearly all killed when exposed at 75°C for 15 minutes. Patzschke (1916) described a *Streptococcus lactis thermophilus* which was killed at 85°C in 10 to 20 minutes, at 80°C in 30 minutes, at 75°C in from 1 to 3 hours, and at 70°C in 5 hours. This species is probably identical with the one described by Orla-Jensen. However, Orla-Jensen's name, being a binomial, is preferred to that suggested by Patzschke. Hammer and Baker (1926) recognize a variety of the species *Streptococcus lactis* which they call *Streptococcus thermophilus* Orla-Jensen.

### TYPE III—*Sarcina lutea* SCHRÖTER, 1872

This species has been isolated much more frequently than any of the others and is recognized by its peculiar heat resistance and its luxuriant bright yellow growth. In liquid media both a tenacious yellow surface pellicle and abundant flocculent sediment appear. Altho similar species have been associated with sterilized milk, Dyar (1895) first described a yellow micrococcus to which he gave the name *Merismopedia flava varians*. Russell and Hastings (1902b) describe it as a micrococcus, the thermal death point of which is 76°C. Ayers and Johnson (1910, 1913) probably saw it among their acid-forming and alkali-forming species which survive the pasteurization of milk. Orla-Jensen (1919) uses the generic term *Tetracoccus*

for organisms of this type. Fenger, Cram, and Rudnick (1920) found it surviving in supposedly sterile surgical sutures and ligatures. More recently, Brannon and Prucha (1927) have observed the unusual heat resistance of this species. The first description of this species was by Schröter (1872) and it is now recognized as *Sarcina lutea*. According to Omeliansky (1911) this species has been isolated from a mastodon carcass in Siberia which had been covered for centuries with ice.

### TYPE IV—*Micrococcus conglomeratus* MIGULA, 1900

The heat resistance of this species is less than that of any of the other five. Ayers and Johnson (1910, 1913), no doubt, encountered it among the bacteria which survive pasteurization. Orla-Jensen uses the generic term *Tetracoccus* for organisms of this type. Hucker (1924) has recognized it as *Micrococcus conglomeratus* Migula.

### TYPE V—*Sarcina rosea* SCHRÖTER, 1872

The isolation of this species is somewhat rare and its heat resistance is usually not recognized. Fenger, Cram, and Rudnick (1920) isolated it in connection with heat resistance studies. Although this species is a typical sarcina, the description by Frankland (1887) of *Micrococcus rosaceus* approaches that of this species as closely as any with the exception of the lack of regular packet formation among the cells. Conn, Esten, and Stocking (1906) describe *Micrococcus lactis rosaeus*, a similar species.

### DESCRIPTION OF SPECIES

Type I. *Microbacterium lacticum* Orla-Jensen, 1919.

Form: Rod, 0.3 to 0.5 by 0.6 to 1.2 microns, occurs singly, in pairs or small irregular clumps of from 4 to 16 individuals, non-motile, Gram-positive.
Spores: Absent.
Agar colonies: Dull grey or white, opaque, raised centers (when not on crowded plates), round, glistening, slow in developing.
Gelatin liquefaction: Negative.
Nitrate reduction: Usually negative.
Litmus milk: Acid only, not coagulated except in old cultures in the presence of heat.
Indole formation: Negative.
Hydrogen sulfide formation: Negative.
Oxygen relations: Aerobe.
Bouillon: Fine sediment.
Carbohydrates: Usually fermented with acid formation. (Details given in Table 12.)
Volatile acid formation: Negative.

Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Negative.
Optimum hydrogen-ion concentration: pH 7.0 to 7.2.
Optimum growth: At ?°C. Laboratory strains grow well. At 30° to 35°C.
Thermal death point: Varies between 80° to 83°C when a 15-hour-old milk culture is heated for 30 minutes. Majority thermal death point is above 74°C for 30 minutes and relatively few, if any, cells are killed at pasteurizing temperatures in milk.

Type II. *Streptococcus thermophilus* Orla-Jensen, 1919.
Form: Coccus, 0.7 to 0.9 micron pointed ends, arranged in pairs usually and sometimes chains of 4 to 6 cells.
Spores: Absent.
Agar colonies: Small, round and white, like those of *Streptococcus lactis* (Lister) Löhnis.
Agar slant: Scanty, bead-like, white.
Gelatin liquefaction: Negative.
Nitrate reduction: Negative.
Litmus milk: Acid and coagulated by freshly isolated cultures in two to four days. Litmus partly reduced sometimes.
Indole formation: Negative.
Hydrogen sulfide formation: Negative.
Oxygen relations: Aerobe.
Bouillon: Fine sediment.
Carbohydrates: Usually fermented with acid formation. (Details given in Table 12.)
Volatile acid formation: Negative.
Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Negative.
Optimum growth: At 40°C.
Thermal death point: Varies between 72° and 74°C in milk culture 15 hours old when heated for 30 minutes. Majority thermal death point is above 65°C in milk for 30 minutes.

Type III. *Sarcina lutea* Schröter, 1872.
Form: Coccus, 1.2 to 1.5 microns in diameter, arranged like a typical sarcina in cubical packets, non-motile, Gram-positive.
Spores: Absent.
Agar colonies: Medium sized, smooth, circular, centers raised, yellow.
Agar slant: Smooth, raised, abundant, yellowish growth.
Gelatin liquefaction: Positive.
Nitrate reduction: Usually positive.
Litmus milk: Acid, coagulated in 4 to 6 days when freshly isolated. Litmus usually partly reduced.
Indole: Usually negative.
Hydrogen sulfide production: Usually negative.
Oxygen relations: Aerobe.
Bouillon: Abundant yellow flocculent sediment. Frequently a surface pellicle develops.

Carbohydrates: Not readily fermented. Medium usually becomes alkalin (Details given in Table 12.)
Volatile acid formation: Slight.
Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Slight increase.
Optimum hydrogen-ion concentration: pH 7.0.
Optimum growth: At 30° to 37°C.
Thermal death point: Varies between 74° and 76°C when a 15-hour-old mi culture is heated for 30 minutes. The majority thermal death point above 68°C for 30 minutes in milk.

Type IV. *Micrococcus conglomeratus* Migula, 1900.
Form: Coccus 0.8 to 1.1 microns in diameter, arranged in irregular mass form tion, non-motile, Gram-positive.
Spores: Absent.
Agar colonies: Moderate, smooth, circular, yellow. Not as abundant growth in Type III.
Agar slant: Moderate, smooth, yellow.
Gelatin liquefaction: Positive.
Nitrate reduction: Positive.
Litmus milk: Acid production only.
Indole formation: Negative.
Hydrogen sulfide formation: Negative.
Oxygen relations: Aerobe.
Bouillon: Abundant yellow sediment.
Carbohydrates: Usually fermented with acid formation. (Details given Table 12.)
Volatile acid formation: Positive.
Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Negative.
Optimum hydrogen-ion concentration: pH 7.0.
Optimum growth: 30° to 37°C.
Thermal death point: Varies from 66° to 68°C when a milk culture 15 hou old is heated for 30 minutes. The majority thermal death point is abo 63°C. This applies to the two cultures under observation and not to t species as a whole.

Type V. *Sarcina rosea* Schröter, 1872.
Form: Coccus, 1.2 to 1.6 microns in diameter, arranged like typical sarcin in cubical packets, non-motile, Gram-positive.
Spores: Absent.
Agar colonies: Medium sized, round, smooth, raised in centers, pink to fl colored.
Agar slant: Moderate, smooth, raised, flesh colored. Growth is slow.
Gelatin liquefaction: Positive.
Nitrate reduction: Usually positive.
Litmus milk: Slight acid formation.
Indole formation: Negative.
Hydrogen sulfide formation: Negative.
Oxygen relations: Aerobe.
Bouillon: Slightly turbid with flesh colored sediment.

Carbohydrates: Not readily fermented. Medium frequently becomes alkaline. (Details given in Table 12.)
Volatile acid formation: Negative.
Ammonia nitrogen formation: Negative.
Amino acid nitrogen formation: Negative or very slight increase.
Optimum growth: 30° to 37°C.
Thermal death point: Varies between 70° and 73°C when a milk culture 15 hours old is heated for 30 minutes. The majority thermal death point is above 67°C when heated for 30 minutes in milk.

## SUMMARY AND CONCLUSIONS

A series of pasteurized milk plates from various cities in this country were sent to the New York State Agricultural Experiment Station for the purpose of studying the so-called "pin-point" flora of pasteurized milk. A few heat-resistant cultures were also sent to the laboratory. A series of heat-resistant cultures were isolated by the author using enrichment methods. The methods consisted of holding samples of raw milk at pasteurizing temperatures and plating hourly for seven hours. A further selection of cultures was made by determining the percentage reduction in the plate count after pasteurization. Where there was more than 90 per cent survival the cultures were retained for further study.

These cultures were divided morphologically into five types, which proved to belong to five distinct species. The utilization of various carbohydrates, some of the higher alcohols, organic acids, and amino acids was determined. Quantitative determinations of volatile acids from milk, d-glucose, lactose, and sucrose were made at the end of two weeks. The amount of ammonia and amino-acid nitrogen and the titratable acid were determined at various times up to 30 days.

Three species, *Microbacterium lacticum* Orla-Jensen (1919), *Streptococcus thermophilus* Orla-Jensen (1919), and *Sarcina lutea* Schröter (1872) were common in milk. Of these three, *Sarcina lutea* could be isolated most frequently. *Micrococcus conglomeratus* Migula (1900) was isolated twice and *Sarcina rosea* Schröter (1872) once. *Microbacterium lacticum* has been developed by enrichment methods in 5 out of 60 samples from individual producers of milk.

The "majority" thermal death points of the above species was above the customary pasteurizing temperatures for milk. The decreasing order of the thermal resistance of the species is as follows: *Microbacterium lacticum*, *Sarcina lutea*, *Streptococcus thermophilus*, *Sarcina rosea*, and *Micrococcus conglomeratus*.

The presence of the above species in market milk as indicated the fermentative studies is not regarded as dangerous to heal Their rates of growth, with the exception of *Streptococcus therm philus*, are relatively slow at temperatures ranging between 2 and 37°C.

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# THERMOPHILIC AND THERMODURIC MICROORGANISMS, WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK

## IV. EFFECT OF AGE OF CULTURE ON THE HEAT RESISTANCE OF NON-SPOREFORMING BACTERIA

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In Cooperation with the
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Young, rapidly growing or adolescent cells of Microbacterium lacticum, Sarcina lutea and Streptococcus thermophilus proved to be more susceptible to high temperatures than the older cells.

The degree of killing at any one age is greater and extends over a longer period of time as the temperatures used approach the thermal death point of the organism.

Pasteurization of milk and food canning in general is most efficient if heat is applied before the microorganisms contained in the milk or food have passed the accelerative growth stage.

# BULLETIN 275: THERMOPHILIC AND THERMODURIC MICROORGANISMS, WITH SPECIAL REFERENCE TO SPECIES ISOLATED FROM MILK[1]

## IV. EFFECT OF AGE OF CULTURE ON THE HEAT RESISTANCE OF NON-SPOREFORMING BACTERIA

A. H. Robertson

Much has been written on the germicidal property of milk and the lag phase in bacterial cultures but few have studied the susceptibility of bacterial cells during the lag and the accelerative growth period. A greater volume of material is available on the susceptibility and reactions of the adult cell to various agents. The purpose of this study is to show the effect of heat on bacterial cells at different periods during the growth of the culture.

The literature on bacterial growth has recently been reviewed by Burke, Sprague and Barnes (1925), Chambers (1920), Sherman and Albus (1923, 1924), Henrici (1926) and Buchanan (1918). Fishoeder (1909) found that anthrax spores after being in a nutrient solution a few minutes (weiniger Minuten) become much more sensitive to heat. The time allowed does not permit germination of the spores. Schultz and Ritz (1910) showed that the thermal resistance of young and old cultures of *B. coli* varied. Cultures three to six hours old were killed by an exposure for 25 minutes at 52° C., while cultures 8 to 24 hours old survived. Sherman and Albus (1923) found that rapidly growing cultures are more susceptible than older cells to exposure to cold (2° C.) and to dilute solutions of both sodium chlorid (2 percent) and phenol (0.5 percent). The older cells are subject to acid agglutination while the younger ones are not. Oerskov (1925) found that young, freshly inoculated cultures are more easily killed than older cells.

### Methods

The organisms studied have been members of the species *Microbacterium lacticum* Orla-Jensen (1919), *Sarcina lutea* Schröter (1872), and *Streptococcus thermophilus* Orla-Jensen (1919). Rapidly growing cultures of the desired organisms were inoculated into 200 c. c.

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portions of sterile milk and incubated at 25° C. Each culture wa plated immediately after inoculation and as frequently thereafter a was necessary. A portion of each sample was pasteurized at 62.8° C for 30 minutes and then plated. The data obtained will indicate th survival of the bacterial culture to heat at different periods during i growth. In order that more information as to the rate of killing migh be obtained, temperatures other than 62.8° C. have been used in severa tests.

## RESULTS

In a series of tables (pages 8 to 24) are given the plate coun before and after heating the milk cultures, and the percentage surviv after heating. The charts on pages 9 to 26 illustrate the general tren of change in the growth curve. The plate counts have been transpose to logarithms and the latter plotted on the vertical scale. The hor zontal scale represents the age of the culture. In some cases, whe the nature of the data would permit, a curve representing the averag curve for two or more species has been constructed. Thus, an averag of the curves for cultures 17, 75 and 98A is presented in chart 4. Th curves for these cultures appear separately in charts 1, 2 and 3, respe tively. These three cultures represent the species *Microbacteriu lacticum*. The majority of the cells of this species will survive heatin for 30 minutes at 62.8° C. at all times during their growth.

Cultures 19, 70, 141Y, 1Z, and 96+ are used to demonstrate th survival at pasteurizing temperatures of a freshly inoculated mi culture of the species *Sarcina lutea*. The growth and the surviv curves for cultures 1Z, 96+, 141Y and 70 are given in charts 5, 6, and 10, respectively. Chart 9 gives the same curves for culture 7 when heated at 71° C. for 30 minutes. The striking early depressio in the survival curve suggests that the young rapidly sub-dividi cells are more easily killed than the adult cells.

Chart 7 is an attempt to show on one chart the growth curve an the two survival curves (at 67° and 71° C.) of culture 19 when su jected to the two different pasteurization temperatures. The pla counts of the unheated culture were so close together that this com parison was permissible.

The species *Streptococcus thermophilus* is represented by cultur 18, 23R, 35, 72, 103 and 123. The growth and the survival curves these respective cultures are illustrated in charts 11, 12, 13, 14, and 16. Because of the similarity of the counts, these data for cu

These survival curves together with those obtained for cultures 19 and 70 at the higher temperatures are convincing of the greater susceptibility of the young rapidly growing cells to heat.

Because cultures of *Streptococcus thermophilus* grew fairly rapidly in milk, an intensive study was made of one culture, 23R, by heating it at 55°, 59°, 62.8°, 67°, 71° and 75° C. Because of the amount of work associated with the determinations at each temperature, only one of the six series could be determined at any one time. The amount of the original inoculum was so controlled that the initial plate counts for the unheated sample would be similar and could be averaged (table 18). From this average a curve has been constructed for the unheated sample (chart 18). On the same chart are shown the general type of the survival curves which may be expected at the six different temperatures. Because no survival was obtained when the culture was heated at 75° C. for 30 minutes except immediately after inoculation, the curve necessarily follows the base line of the diagram and, hence, can not be illustrated on the chart.

In order to illustrate these same facts from the standpoint of percentages killed, chart 19 has been constructed. Because of the small survival at 71° C. and no survival at 75° C. the curves for these two determinations practically coincide with the 100 percent killed line at the top of the diagram.

## DISCUSSION

No extended lag phase was observed. A milk culture of young rapidly growing cells, usually 7 to 10 hours old, was used for inoculating the samples which were to receive careful observation. The use of such cultures tends to eliminate the lag phase (Sherman and Albus, 1924). The regressive or death phase did not develop to any appreciable extent in the milk cultures even on the fourth day. Possibly the milk may exert a protective action against the death phase of the cell which is not so pronounced in broth cultures of the same age.

The survival curve when culture 23R (chart 18) is heated at 55° C. drops rapidly. At the end of the second hour of growth 79.5 percent of the bacteria were killed, at the end of the fourth hour 93 percent and thereafter a relatively small percent were killed. When heated to a temperature of 59° C., 96 percent were killed when the culture was two hours old, 99.9 percent when four and six hours, 81.6 percent when eight hours and a relatively small percentage thereafter. As higher temperatures are used, an increasing death rate occurs which extends over a longer period each time until at the end of

the sixth hour when heated at 62.8° C., no colonies appeared on the plate. At the end of the 10th hour colonies reappeared and after the 16th hour relatively few of the cells were killed. The survival curve declines more suddenly with increased temperatures and finally ceases to approach the growth curve for the unheated cells. For instance at the end of the 47th and 81st hours when the cultures were heated to 71° C., 99.8 and 99 percent of the cells were killed. When a temperature of 75° C. was used the survival curve did not reappear even at the 71st hour. Therefore, the true thermal death point of culture 23R is somewhere between 71° and 75° C., when a milk culture of the organism is heated for 30 minutes.

In the commercial pasteurization of milk where the milk has not been properly cooled before heating and is over 20 hours old, it is quite possible that, even in spite of a 99 percent reduction in bacterial count frequently 100,000 bacteria may still be left in the milk. However if the same milk could have been pasteurized before the end of the accelerative growth phase, the bacterial content in most cases would be reduced by 99 percent over what would have been the case if the milk had been pasteurized later. In commercial pasteurization, the bacterial content, unless spore formers are abundant, is composed largely of cells with less resistance to heat than those which have been discussed in this work.

When culture 18, pasteurized at 62.8° C. for 30 minutes, was two hours old, the reduction was from 9,000 per c. c. to 500 per c. c., when four hours old from 25,000 per c. c. to 1,000 per c. c., when six hours old from 179,000 per c. c. to less than 100 per c. c., when eight hours old from 1,360,000 per c. c. to less than 100 per c. c., when ten hours old from 5,100,000 per c. c. to less than 1,000 per c. c., but when 1 hours old the reduction was from 403,000,000 to 193,000,000 per c. and when 71 hours old from 1,740,000,000 to 1,370,000,000 per c. c.

Obviously most effective pasteurization of milk can be secured during the early stages of the accelerative growth period. A similar timely application of heat before the accelerative bacterial growth rate begins to decline might well be applied to all canning and preserving operations where heat is used to destroy the microorganisms.

The bacterial growth curves are convincing of the rapid rate of reproduction of bacteria under optimum growth conditions in milk between the 3rd and 10th hours after milking. After the 10th hour of bacterial growth, the numbers are so great that the addition of several millions, more or less, does not materially alter the sanitary

quality of the milk. Fundamentally, these data support the demands which milk companies and public health officials are exercising to improve milk quality. The necessity for prolonging the lag phase of bacterial growth by proper cooling of the milk is strikingly manifested.

The so-called germicidal property of milk (Stocking, 1904, and Chambers, 1920) and the toxin accumulation theory of Chesney (1916), the allalocatalytic force of Robertson (1923) and the adjustment period of Sherman and Albus (1924) may be explained as some form of a lowered resistance or greater susceptibility of the adolescent cells before they reach the mature stage. The more severe the devitalizing agent, the greater will be the numbers killed and the longer the period will be during which the destructive influence is active. The less severe the antagonizing agency, the sooner will the process of adaptation or adjustment be completed.

## SUMMARY

Milk cultures of *Microbacterium lacticum, Sarcina lutea* and *Streptococcus thermophilus* were pasteurized for 30 minutes at several different temperatures, 62.8° C. being the temperature most commonly used. Sub-samples of the original culture were pasteurized immediately after inoculation with the above cultures and at such times later as desired. Plate counts were made both before and after pasteurization in order to determine the numbers killed.

Data are presented and charts have been constructed to illustrate the general trend of the curves both for the growth of the culture and its susceptibility to the pasteurization temperatures employed.

## CONCLUSIONS

The young, rapidly growing or adolescent cells are more susceptible than the older cells to the killing action of high temperatures. As the temperatures used approach the thermal death points of the organisms the degree of killing at any one age is greater and extends over a longer period of time.

Most efficient pasteurization of milk or canning of foods can be obtained if the heat is applied to the substances before the microorganisms have passed the accelerative growth stage.

## PLATE COUNTS BEFORE AND AFTER THE PASTEURIZATION OF PURE MILK CULTURES (PAGES 8-26)

Subsamples of the original milk were pasteurized at different ag during the growth of the culture to determine whether the heat resi ance of the growing cells was uniform.

TABLE 1—*Microbacterium lacticum*, CULTURE 17, PASTEURIZD AT 62.8° C. 30 MINUTES

| Age of culture in hours | Plate counts | | Percentag survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 4,720 | 3,250 | 68.9 |
| 8 | 321,000 | 340,000 | 105.9 |
| 10 | 322,000 | 617,000 | 191.6 |
| 12 | 740,000 | 670,000 | 90.5 |
| 14 | 980,000 | 880,000 | 89.9 |
| 16 | 887,000 | 780,000 | 87.9 |
| 20 | 1,470,000 | 1,250,000 | 85.0 |
| 33 | 1,870,000 | 1,730,000 | 92.5 |
| 36 | 8,630,000 | 8,730,000 | 101.2 |
| 43 | ..... | 44,000,000 | ... |
| 56 | 78,700,000 | 63,000,000 | 80.0 |
| 59 | ..... | ..... | ... |
| 63 | ..... | ..... | ... |
| 81 | 162,000,000 | 105,000,000 | 64.8 |

TABLE 2—*Microbacterium lacticum*, CULTURE 75, PASTEURIZED AT 62.8° C. 30 MINUTES

| Age of culture in hours | Plate counts | | Percentag survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 167,000 | 166,000 | 99.4 |
| 8 | 1,270,000 | 66,700 | 0.6 |
| 10 | 3,430,000 | 1,630,000 | 47.5 |
| 12 | 16,600,000 | 2,830,000 | 17.0 |
| 14 | 26,300,000 | 19,300,000 | 73.4 |
| 16 | 46,000,000 | ..... | ... |
| 20 | 304,000,000 | ..... | ... |
| 33 | 331,000,000 | 326,000,000 | 98.5 |
| 36 | 386,000,000 | 630,000,000 | 163.3 |
| 43 | ..... | 1,470,000,000 | ... |
| 56 | 1,220,000,000 | 1,360,000,000 | 111.5 |
| 59 | 1,540,000,000 | 410,000,000 | 26.6 |
| 63 | 2,350,000,000 | 1,290,000,000 | 54.9 |
| 81 | 1,300,000,000 | 1,460,000,000 | 112.3 |

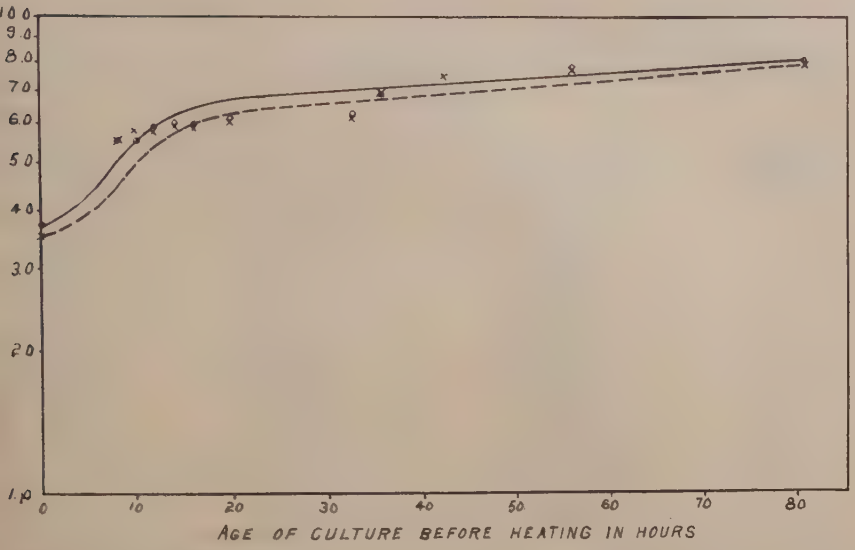


CHART 1.—*Microbacterium lacticum*, culture 17, pasteurized at 62.8° C for 30 minutes.

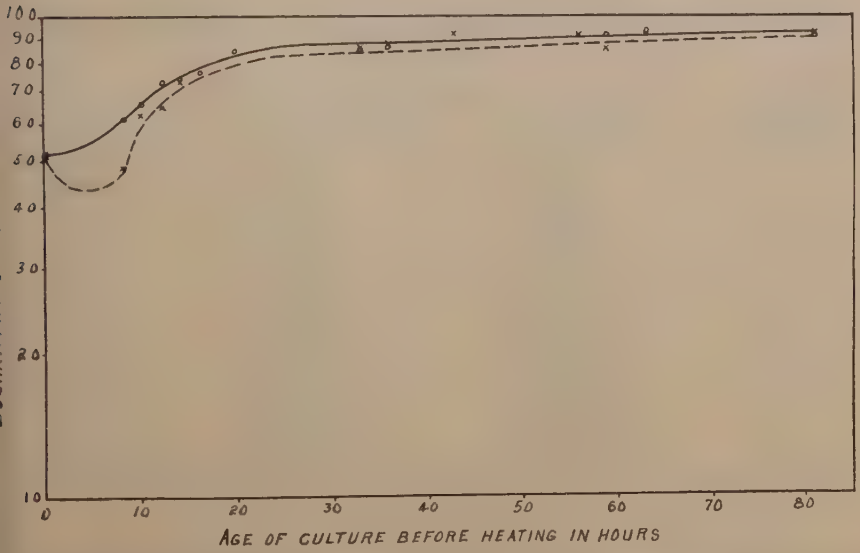


CHART 2.—*Microbacterium lacticum*, culture 75, pasteurized at 62.8° C. for 30 minutes.

TABLE 3—*Microbacterium lacticum*, CULTURE 98A, PASTEURIZED AT 62.8° C. FO
30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 26,000 | 22,200 | 84.6 |
| 8 | 316,000 | 241,000 | 76.3 |
| 10 | 513,000 | 410,000 | 79.9 |
| 12 | 870,000 | 780,000 | 89.7 |
| 14 | 1,220,000 | 1,310,000 | 107.3 |
| 16 | 2,760,000 | 2,460,000 | 89.1 |
| 20 | 8,100,000 | 7,230,000 | 89.3 |
| 33 | 24,700,000 | 26,200,000 | 106.1 |
| 36 | 51,000,000 | 54,000,000 | 105.8 |
| 43 | 104,000,000 | 111,000,000 | 106.7 |
| 56 | 610,000,000 | 572,000,000 | 93.8 |
| 59 | 1,020.000,000 | 1,240,000,000 | 121.5 |
| 63 | 1,460,000,000 | 1,370,000,000 | 93.8 |
| 81 | 2,530,000,000 | 1,620,000,000 | 64.0 |

TABLE 4—PLATE COUNTS OF CULTURES 17, 75, AND 98A, AVERAGED FROM THR
PRECEDING TABLES

| Age of culture in hours | Plate counts | | Percentag survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 65,900 | 63,800 | 96.8 |
| 8 | 636,000 | 216,000 | 34.0 |
| 10 | 1,420,000 | 886,000 | 62.4 |
| 12 | 6,070,000 | 1,430,000 | 23.6 |
| 14 | 9,500,000 | 7,160,000 | 75.4 |
| 16 | 16,500,000 | 11,100,000 | 67.3 |
| 20 | 104,000,000 | 86,200,000 | 82.9 |
| 33 | 119,000,000 | 127,000,000 | 106.7 |
| 36 | 148,000,000 | 231,000,000 | 156.1 |
| 43 | 104,000,000 | 111,000,000 | 106.7 |
| 56 | 636,000,000 | 665,000,000 | 104.6 |
| 59 | 1,280,000,000 | 825,000,000 | 64.4 |
| 63 | 1,910,000,000 | 1,330,000,000 | 69.6 |
| 81 | 1,330,000,000 | 1,060,000,000 | 79.7 |

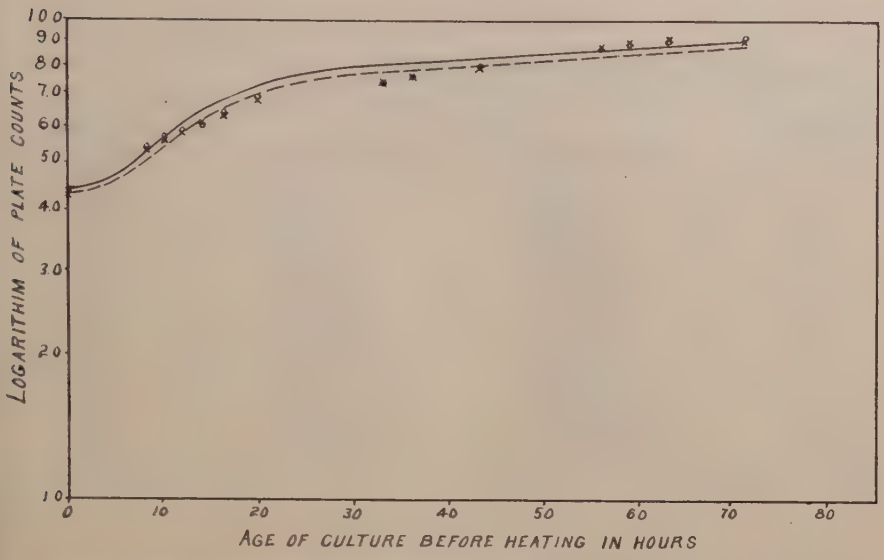


CHART 3.—*Microbacterium lacticum*, culture 98A, pasteurized at 62.8° C. for 30 minutes.

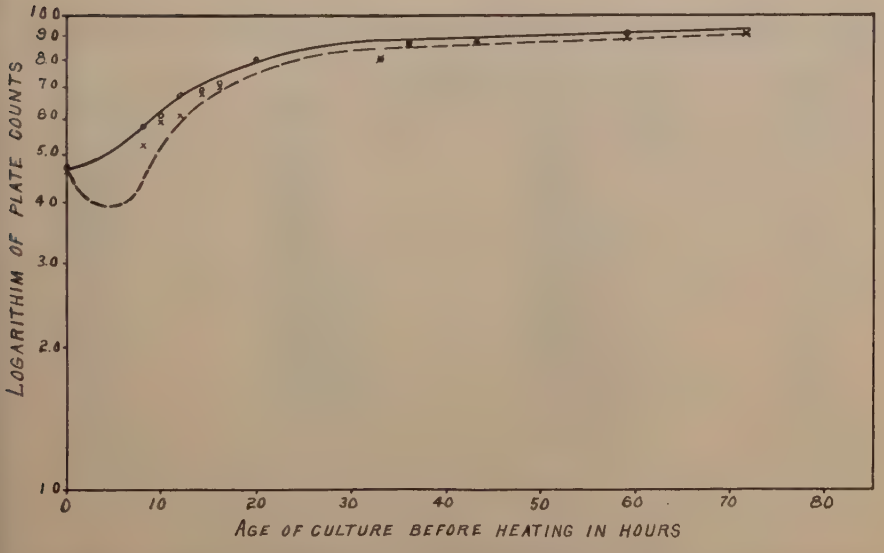


CHART 4.—Curves representing the averaged curves from the three preceding charts of cultures 17, 75 and 98A, *Microbacterium lacticum*.

TABLE 5—*Sarcina lutea*, CULTURE 1Z, PASTEURIZED AT 62.8° C. FOR 30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 141,000 | 130,000 | 92.8 |
| 8 | 410,000 | 175,000 | 42.7 |
| 10 | 1,000,000 | 505,000 | 50.5 |
| 12 | 2,000,000 | 1,420,000 | 71.0 |
| 15 | 5,100,000 | 7,500,000 | 147.1 |
| 21 | 15,400,000 | 18,000,000 | 115.6 |
| 33 | 87,000,000 | 94,000,000 | 108.0 |
| 36 | 152,000,000 | 108,000,000 | 71.0 |
| 39 | 137,000,000 | 141,000,000 | 102.9 |
| 43 | 167,000,000 | 164,000,000 | 98.2 |
| 57 | 242,000,000 | 168,000,000 | 69.4 |
| 61 | 267,000,000 | 216,000,000 | 83.3 |
| 79 | 280,000,000 | 249,000,000 | 88.9 |

TABLE 6—*Sarcina lutea*, CULTURE 96+, PASTEURIZED AT 62.8° C. FOR 30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 5,300 | 3,500 | 66.0 |
| 8 | 17,900 | 13,900 | 77.6 |
| 10 | 37,000 | 28,000 | 75.7 |
| 12 | 350,000 | 314,000 | 89.7 |
| 15 | 1,900,000 | 1,620,000 | 85.3 |
| 21 | 4,500,000 | 4,270,000 | 94.7 |
| 33 | 9,100,000 | 7,300,000 | 80.2 |
| 36 | 17,200,000 | 13,000,000 | 75.6 |
| 39 | 18,500,000 | 22,600,000 | 121.1 |
| 43 | 29,400,000 | 27,600,000 | 93.9 |
| 57 | 142,000,000 | 120,000,000 | 84.5 |
| 61 | 171,000,000 | 156,000,000 | 91.2 |
| 79 | 241,000,000 | 249,000,000 | 103.3 |

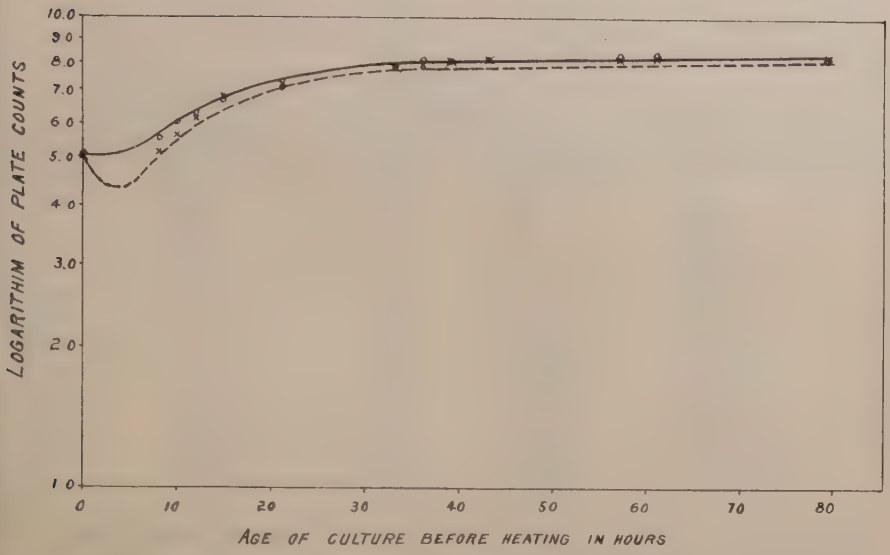


CHART 5.—*Sarcina lutea*, culture 1Z, pasteurized at 62.8° C. for 30 minutes.

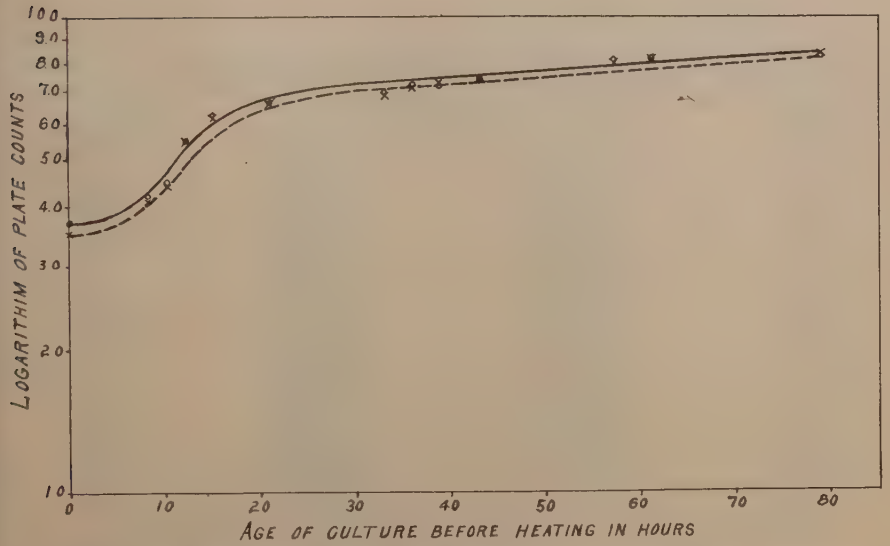


CHART 6.—*Sarcina lutea*, culture 96+, pasteurized at 62.8° C. for 30 minutes.

TABLE 7—*Sarcina lutea*, CULTURE 19, PASTEURIZED AT 67° AND 71° C. FOR 30 MINUT

| ge of ulture hours | Averaged plate count before heating* | Pasteurized at 67° C. for 30 minutes | | Pasteurized at 71° C. for 30 minutes | |
|---|---|---|---|---|---|
| | | Plate count after heating | Percentage survived | Plate count after heating | Percenta survive |
| 0 | 4,020 | 2,110 | 52.5 | 4,100 | 101.9 |
| 2 | 5,440 | 760 | 14.0 | 240 | 4.4 |
| 4 | 8,100 | <100 | 1.2 | <100 | 1.2 |
| 6 | 14,400 | <100 | 0.7 | <100 | 0.7 |
| 8 | 28,800 | 3,410 | 11.8 | <100 | 0.3 |
| 10 | 58,000 | 16,000 | 27.6 | <100 | <0.1 |
| 12 | 116,000 | 122,000 | 105.1 | <1,000 | 0.9 |
| 16 | 159,000 | 152,000 | 95.6 | <1,000 | 0.7 |
| 26 | 237,000 | 287,000 | 121.1 | 178,000 | 75.1 |
| 30 | 250,000 | 263,000 | 105.2 | 222,000 | 88.8 |
| 34 | 295,000 | 324,000 | 109.8 | 288,000 | 97.6 |
| 47 | 394,000 | 372,000 | 94.4 | 346,000 | 87.6 |
| 71 | 607,000 | 577,000 | 95.0 | 688,000 | 113.3 |
| 95 | 2,060,000 | 1,570,000 | 76.2 | 2,070,000 | 100.5 |

*Plate counts of corresponding unheated samples at the two different temperatures avera

TABLE 8—*Sarcina lutea*, CULTURE 70, PASTEURIZED AT 62.8° C. FOR 30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 377,000 | 311,000 | 82.5 |
| 8 | 1,560,000 | 1,240,000 | 79.5 |
| 10 | 2,420,000 | 2,380,000 | 98.3 |
| 12 | 6,400,000 | 6,200,000 | 96.9 |
| 15 | 11,900,000 | 8,900,000 | 74.9 |
| 21 | 13,300,000 | 14,900,000 | 112.0 |
| 33 | 26,700,000 | 27,400,000 | 102.2 |
| 36 | 22,800,000 | 25,300,000 | 110.9 |
| 39 | 31,200,000 | 30,500,000 | 97.7 |
| 43 | 35,300,000 | 43,000,000 | 121.8 |
| 57 | 78,000,000 | 70,000,000 | 98.9 |
| 59 | 84,000,000 | 83,000,000 | 101.2 |
| 61 | 84,000,000 | 86,000,000 | 102.4 |
| 79 | 230,000,000 | 228,000,000 | 99.1 |
| 83 | 223,000,000 | 248,000,000 | 111.2 |

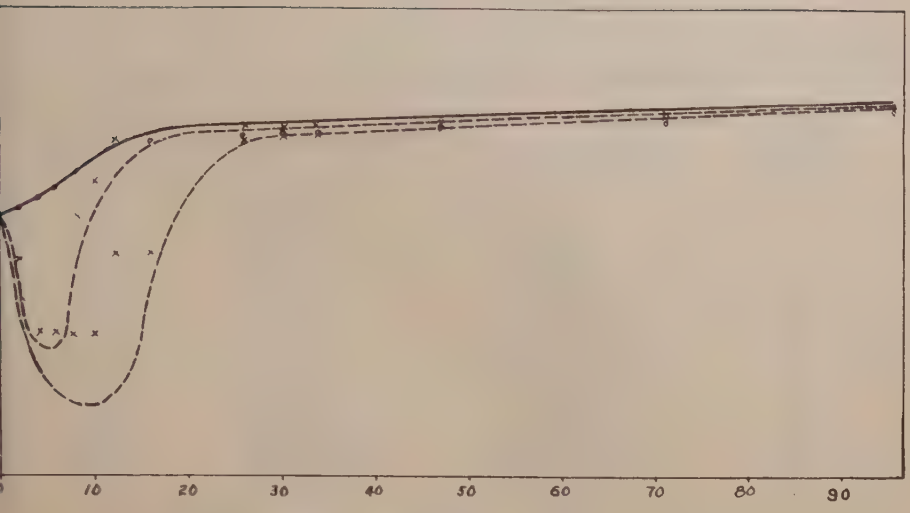


CHART 7.—*Sarcina lutea*, culture 19, pasteurized at 67° and 71° C. for 30 minutes. The lower broken line illustrates the general trend of the curve where the higher temperature is employed.

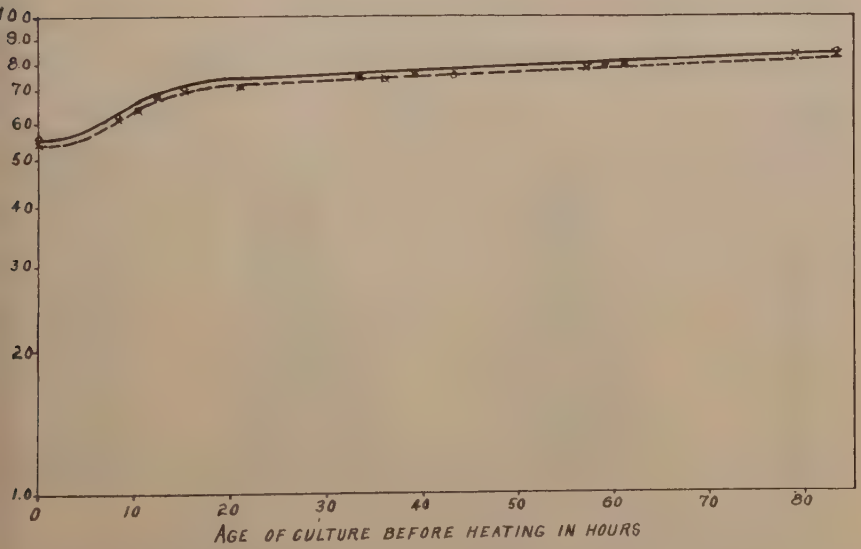


CHART 8.—*Sarcina lutea*, culture 70, pasteurized at 62.8° C. for 30 minutes.

TABLE 9—*Sarcina lutea*, CULTURE 70, PASTEURIZED AT 71° C. FOR 30 MINUTE

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 41,300 | 38,700 | 93.7 |
| 2 | 61,000 | 3,400 | 5.6 |
| 4 | 178,000 | <100 | <0.1 |
| 6 | 201,000 | <100 | <0.1 |
| 8 | 684,000 | <100 | <0.1 |
| 10 | 1,140,000 | <1,000 | <0.1 |
| 12 | 2,160,000 | <1,000 | <0.1 |
| 16 | 7,100,000 | 58,000 | 0.8 |
| 26 | 30,000,000 | 11,600,000 | 38.7 |
| 30 | 38,000,000 | 36,000,000 | 94.0 |
| 34 | 53,000,000 | 57,000,000 | 107.5 |
| 47 | 112,000,000 | 122,000,000 | 108.9 |
| 71 | 210,000,000 | 231,000,000 | 110.0 |
| 95 | 276,000,000 | 247,000,000 | 89.5 |

TABLE 10—*Sarcina lutea*, CULTURE 141Y, PASTEURIZED AT 62.8° C. FOR 30 MINUTE

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 180,000 | 134,000 | 74.4 |
| 8 | 296,000 | 19,300 | 6.5 |
| 10 | 693,000 | 148,000 | 21.4 |
| 12 | 1,970,000 | 1,530,000 | 77.7 |
| 15 | 3,750,000 | 3,770,000 | 100.5 |
| 21 | 12,200,000 | 12,700,000 | 104.1 |
| 33 | 276,000,000 | 190,000,000 | 68.8 |
| 36 | 205,000,000 | 245,000,000 | 119.5 |
| 39 | 260,000,000 | 268,000,000 | 103.1 |
| 43 | 283,000,000 | 271,000,000 | 95.8 |
| 57 | 406,000,000 | 352,000,000 | 86.7 |
| 61 | 367,000,000 | 363,000,000 | 98.8 |
| 79 | 435,000,000 | 469,000,000 | 107.8 |
| 83 | 479,000,000 | 454,000,000 | 94.8 |

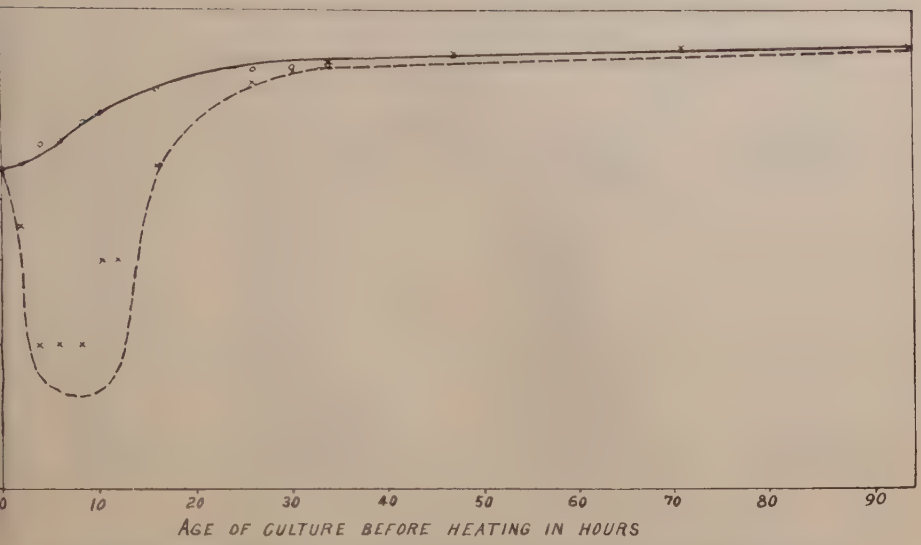


CHART 9.—*Sarcina lutea*, culture 70, pasteurized at 71° C. for 30 minutes.

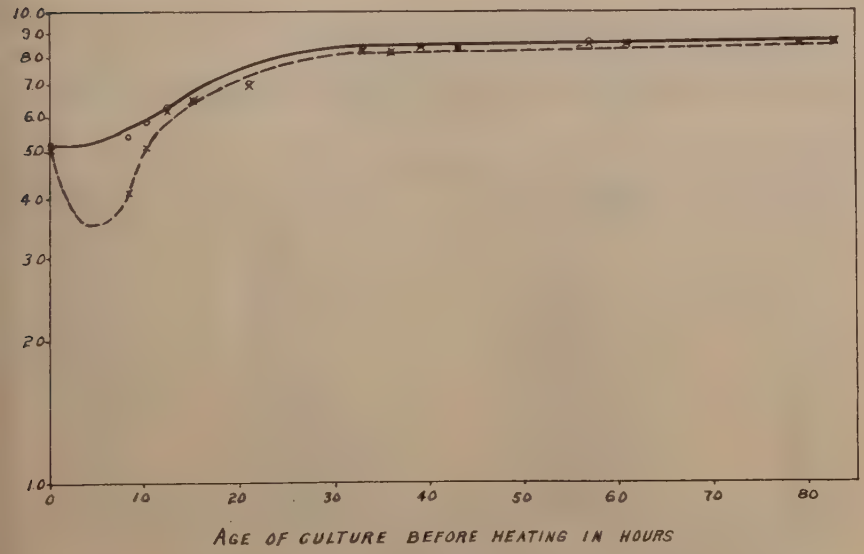


CHART 10.—*Sarcina lutea*, culture 141Y, pasteurized at 62.8° C. for 30 minutes.

TABLE 11—*Streptococcus thermophilus*, CULTURE 18, PASTEURIZED AT 62.8° C. FO
30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 6,300 | 5,300 | 84.1 |
| 2 | 9,000 | 500 | 5.6 |
| 4 | 25,000 | 1,000 | 4.0 |
| 6 | 179,000 | <100 | <0.1 |
| 8 | 1,360,000 | <100 | <0.1 |
| 10 | 5,100,000 | <1,000 | <0.1 |
| 14 | 30,500,000 | <1,000 | <0.1 |
| 16 | 79,000,000 | <1,000 | <0.1 |
| 26 | 403,000,000 | 193,000,000 | 47.9 |
| 30 | 500,000,000 | 260,000,000 | 52.0 |
| 34 | 580,000,000 | 340,000,000 | 58.6 |
| 47 | 1,220,000,000 | 840,000,000 | 68.8 |
| 71 | 1,740,000,000 | 1,370,000,000 | 78.7 |

TABLE 12—*Streptococcus thermophilus*, CULTURE 23R, PASTEURIZED AT 62.8° C
FOR 30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 4,900 | 3,500 | 71.4 |
| 2 | 10,000 | <100 | <1.0 |
| 4 | 23,500 | <100 | <0.4 |
| 6 | 162,000 | <100 | <0.1 |
| 8 | 1,170,000 | <100 | <0.1 |
| 10 | 3,850,000 | <1,000 | <0.1 |
| 14 | 29,400,000 | <1,000 | <0.1 |
| 16 | 87,000,000 | <1,000 | <0.1 |
| 26 | 580,000,000 | 89,000,000 | 15.3 |
| 30 | 700,000,000 | 189,000,000 | 27.0 |
| 34 | 1,090,000,000 | 200,000,000 | 18.3 |
| 47 | 690,000,000 | 570,000,000 | 82.6 |
| 71 | 630,000,000 | 410,000,000 | 65.1 |

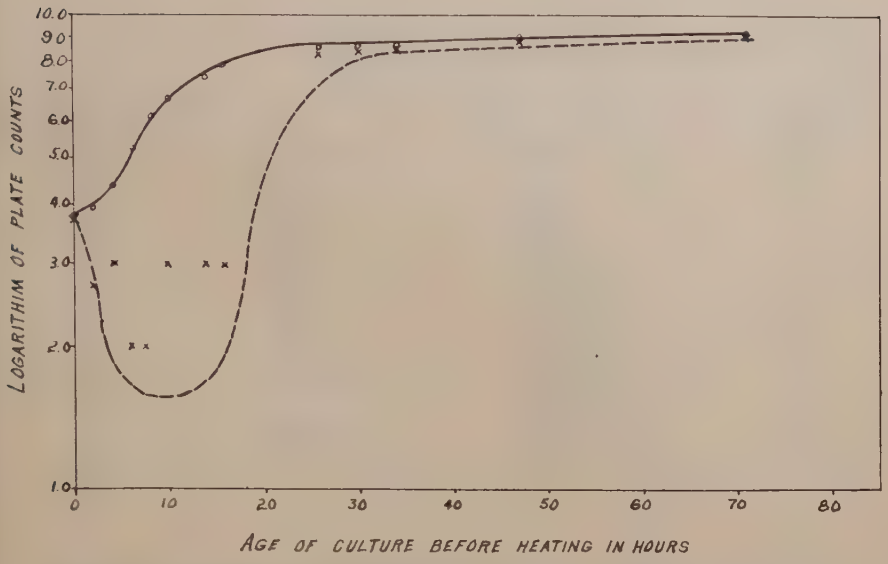


CHART 11.—*Streptococcus thermophilus*, culture 18, pasteurized at 62.8° C. for 30 minutes.

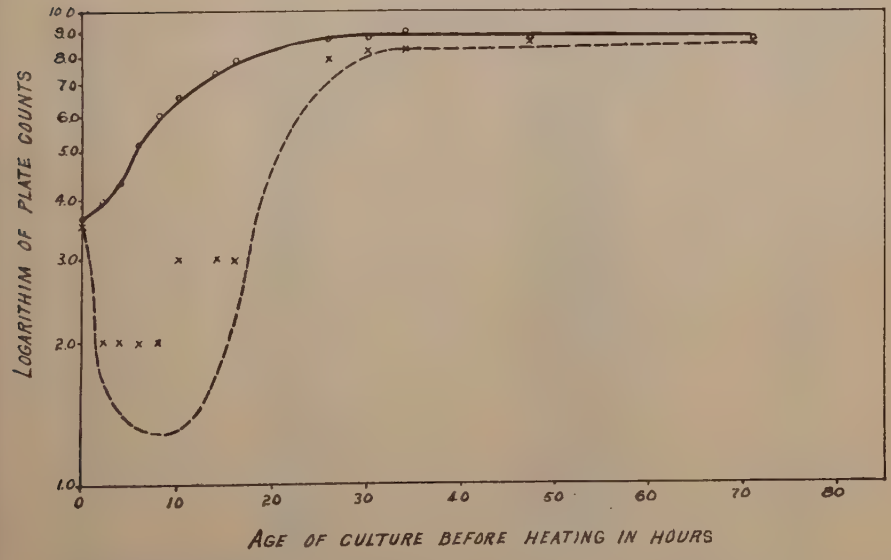


CHART 12.—*Streptococcus thermophilus*, culture 23R, pasteurized at 62.8° C. for 30 minutes.

TABLE 13—*Streptococcus thermophilus*, CULTURE 35, PASTEURIZED AT 62.8° C. FOR 30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 3,500 | 2,900 | 82.8 |
| 2 | 8,900 | 530 | 6.0 |
| 4 | 48,000 | <100 | <0.1 |
| 6 | 260,000 | <100 | <0.1 |
| 8 | 4,430,000 | <100 | <0.1 |
| 10 | 25,700,000 | <1,000 | <0.1 |
| 14 | 233,000,000 | 52,000 | <0.1 |
| 16 | 286,000,000 | 53,000 | <0.1 |
| 26 | 477,000,000 | 34,000,000 | 7.1 |
| 30 | 390,000,000 | 74,300,000 | 19.0 |
| 34 | 403,000,000 | ..... | ... |
| 47 | 2,350,000,000 | 1,980,000,000 | 84.2 |
| 71 | 4,900,000,000 | 1,560,000,000 | 31.8 |

TABLE 14—*Streptococcus thermophilus*, CULTURE 72, PASTEURIZED AT 62.8° C. FOR 30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 254,000 | 207,000 | 81.5 |
| 1 | 335,000 | 184,000 | 54.9 |
| 2 | 486,000 | 19,700 | 4.1 |
| 3 | 817,000 | <1,000 | <0.1 |
| 4 | 1,500,000 | <1,000 | <0.1 |
| 5 | 5,200,000 | <1,000 | <0.1 |
| 6 | 7,770,000 | <1,000 | <0.1 |
| 7 | 16,400,000 | <1,000 | <0.1 |
| 8 | 29,700,000 | <1,000 | <0.1 |
| 9 | 36,300,000 | <1,000 | <0.1 |
| 10 | 65,000,000 | <1,000 | <0.1 |
| 11 | 109,000,000 | <1,000 | <0.1 |
| 12 | 75,000,000 | <1,000 | <0.1 |
| 13 | 324,000,000 | <1,000 | <0.1 |
| 25 | 417,000,000 | 3,300,000 | 0.8 |
| 28 | 387,000,000 | 45,300,000 | 1.2 |
| 31 | 433,000,000 | 97,000,000 | 22.4 |
| 35 | 600,000,000 | 95,000,000 | 15.8 |
| 49 | 561,000,000 | ..... | ... |
| 54 | 611,000,000 | 475,000,000 | 77.7 |
| 73 | 669,000,000 | 497,000,000 | 74.3 |

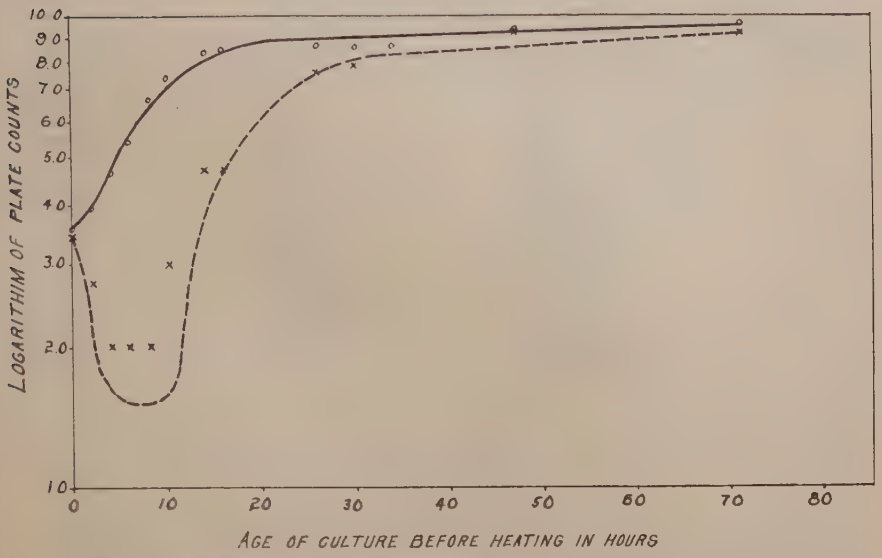


CHART 13.—*Streptococcus thermophilus*, culture 35, pasteurized at 62.8° C. for 30 minutes.

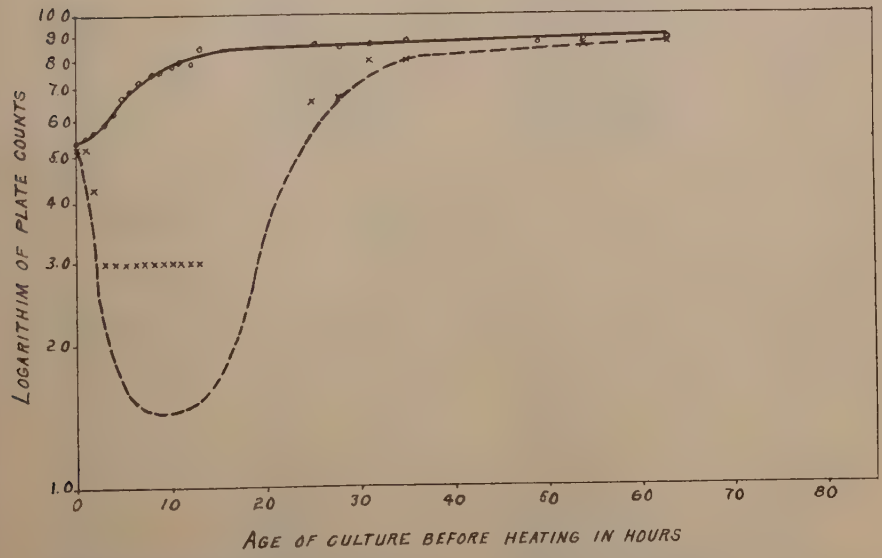


CHART 14.—*Streptococcus thermophilus*, culture 72, pasteurized at 62.8° C. for 30 minutes.

TABLE 15—*Streptococcus thermophilus*, CULTURE 103, PASTEURIZED AT 62.8° C. FOR 30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 6,300 | 5,800 | 92.1 |
| 2 | 19,200 | <100 | <0.5 |
| 4 | 88,000 | <100 | <0.1 |
| 6 | 950,000 | <100 | <0.1 |
| 8 | 10,200,000 | <1,000 | <0.1 |
| 10 | 34,000,000 | <1,000 | <0.1 |
| 14 | 260,000,000 | <1,000 | <0.1 |
| 16 | 400,000,000 | 1,130,000 | <0.1 |
| 26 | 570,000,000 | 420,000,000 | 0.2 |
| 30 | 570,000,000 | 440,000,000 | 73.7 |
| 34 | 553,000,000 | 460,000,000 | 83.2 |
| 47 | 520,000,000 | 410,000,000 | 78.8 |
| 71 | 3,600,000,000 | ..... | ... |

TABLE 16—*Streptococcus thermophilus*, CULTURE 123, PASTEURIZED AT 62.8° C. FOR 30 MINUTES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 19,000 | 8,900 | 46.8 |
| 2 | 233,000 | <100 | <0.1 |
| 4 | 610,000 | <100 | <0.1 |
| 6 | 1,620,000 | <100 | <0.1 |
| 8 | 8,300,000 | <1,000 | <0.1 |
| 12 | 41,000,000 | <1,000 | <0.1 |
| 14 | 72,000,000 | 10,000 | <0.1 |
| 24 | 441,000,000 | 94,000,000 | 21.3 |
| 28 | 1,910,000,000 | 143,000,000 | 7.5 |
| 32 | 2,500,000,000 | 251,000,000 | 9.9 |
| 43 | 4,540,000,000 | 1,650,000,000 | 36.3 |
| 71 | 6,300,000,000 | 410,000,000 | 6.5 |

TABLE 17—PLATE COUNTS OF CULTURES 18, 23R, 35 AND 103, AVERAGED FROM PRECEDING TABLES

| Age of culture in hours | Plate counts | | Percentage survived |
|---|---|---|---|
| | Before heating | After heating | |
| 0 | 5,250 | 4,380 | 88.4 |
| 2 | 11,800 | 308 | 2.6 |
| 4 | 46,200 | 425 | 0.9 |
| 6 | 388,000 | <100 | <0.1 |
| 8 | 4,290,000 | 425 | <0.1 |
| 10 | 17,200,000 | <1,000 | <0.1 |
| 14 | 138,000,000 | 113,000 | <0.1 |
| 16 | 213,000,000 | 296,000 | <0.1 |
| 26 | 508,000,000 | 184,000,000 | 36.2 |
| 30 | 540,000,000 | 241,000,000 | 44.6 |
| 34 | 657,000,000 | 333,000,000 | 50.7 |
| 47 | 1,200,000,000 | 950,000,000 | 79.2 |
| 71 | 2,470,000,000 | 585,000,000 | 23.7 |

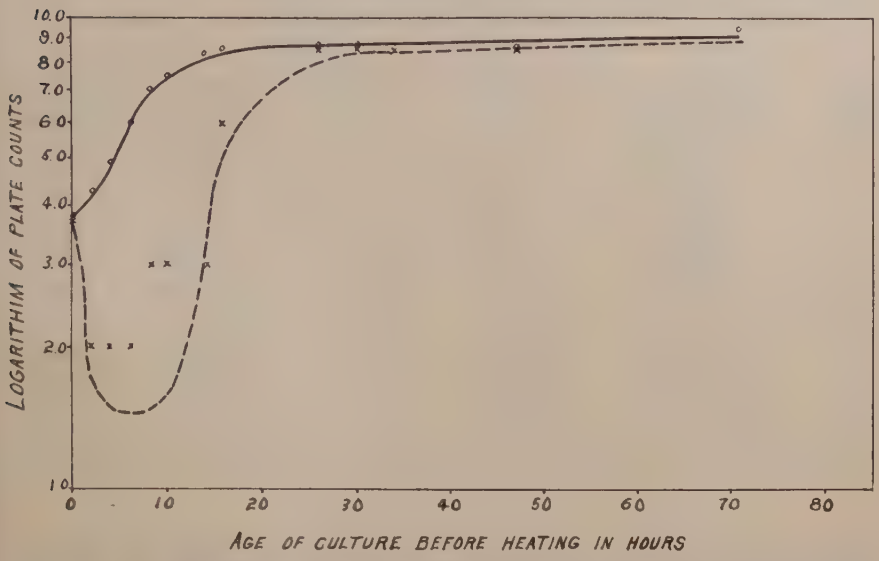


CHART 15.—*Streptococcus theromphilus*, culture 103, pasteurized at 62.8° C. for 30 minutes.

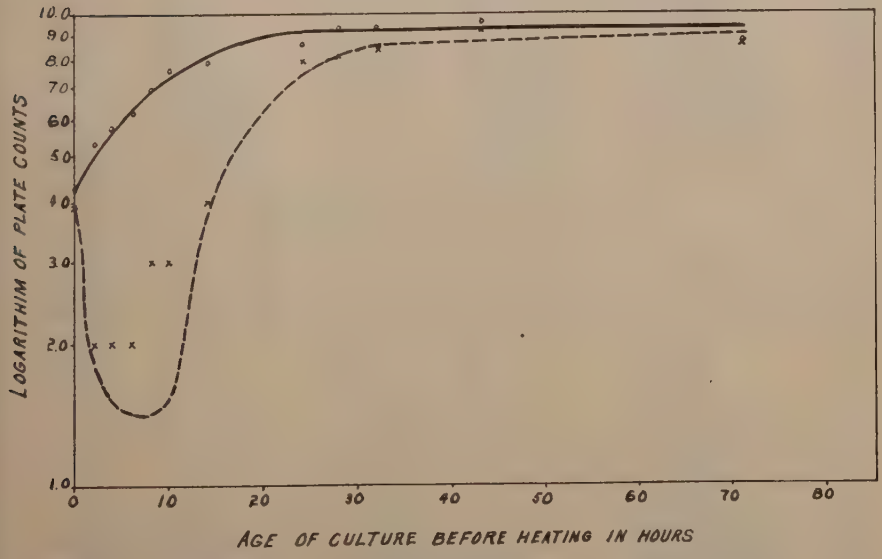


CHART 16.—*Streptococcus thermophilus*, culture 123, pasteurized at 62.8° C. for 30 minutes.

TABLE 18—*Streptococcus thermophilus*, CULTURE 23R, PASTEURIZED AT 55°, 59°, 62.8°, 67°, 71°, AND 75° C. FOR 30 MINUTES

| Age of culture in hours | Averaged plate count before heating* | *Streptococcus thermophilus*, culture 23R, heated for 30 minutes at 55° C. | | 59° C. | |
|---|---|---|---|---|---|
| | | Plate count after heating | Percentage survived | Plate count after heating | Percentage survived |
| 0 | 15,300 | 9,870 | 64.51 | 15,300 | 100.00 |
| 2 | 29,800 | 6,100 | 20.47 | 1,300 | 4.36 |
| 4 | 74,000 | 5,230 | 7.07 | 43 | 0.06 |
| 6 | 229,000 | 151,000 | 65.94 | 61 | 0.03 |
| 8 | 912,000 | 1,340,000 | 146.92 | 168,000 | 18.42 |
| 10 | 2,570,000 | 3,080,000 | 119.84 | 1,740,000 | 67.70 |
| 14 | 24,000,000 | 27,200,000 | 113.33 | 19,300,000 | 79.42 |
| 16 | 47,000,000 | 39,600,000 | 84.25 | 46,700,000 | 0.36 |
| 26 | 534,000,000 | 501,000,000 | 93.82 | 314,000,000 | 58.80 |
| 30 | 730,000,000 | 730,000,000 | 100.00 | 653,000,000 | 89.45 |
| 34 | 1,100,000,000 | 967,000,000 | 87.91 | 843,000,000 | 76.64 |
| 47 | 1,630,000,000 | 1,720,000,000 | 105.52 | 1,370,000,000 | 84.05 |
| 71 | 1,560,000,000 | 1,780,000,000 | 114.10 | 1,580,000,000 | 101.28 |

| Age of culture in hours | Averaged plate count before heating* | *Streptococcus thermophilus*, culture 23R, heated for 30 minutes at 62.8° C. | | 67° C. | |
|---|---|---|---|---|---|
| | | Plate count after heating | Percentage survived | Plate count after heating | Percentage survived |
| 0 | 15,300 | 9,200 | 60.13 | 15,700 | 102.61 |
| 2 | 29,800 | 131 | 0.44 | 217 | 0.73 |
| 4 | 74,000 | 17 | 0.02 | 17 | 0.02 |
| 6 | 229,000 | 0 | <0.01 | 0 | <0.01 |
| 8 | 912,000 | 0 | <0.01 | 0 | <0.01 |
| 10 | 2,570,000 | 21 | <0.01 | 0 | <0.01 |
| 14 | 24,000,000 | 247 | <0.01 | 30 | <0.01 |
| 16 | 47,000,000 | 2,840,000 | 0.60 | 140 | <0.01 |
| 26 | 534,000,000 | 373,000,000 | 69.85 | 3,160 | <0.01 |
| 30 | 730,000,000 | 643,000,000 | 88.08 | 583,000,000 | 79.86 |
| 34 | 1,100,000,000 | 817,000,000 | 74.27 | 617,000,000 | 56.09 |
| 47 | 1,630,000,000 | 887,000,000 | 54.42 | 847,000,000 | 51.96 |
| 71 | 1,560,000,000 | 853,000,000 | 54.68 | 977,000,000 | 62.63 |

| Age of culture in hours | Averaged plate count before heating* | *Streptococcus thermophilus*, culture 23R, heated for 30 minutes at 71° C. | | 75° C. | |
|---|---|---|---|---|---|
| | | Plate count after heating | Percentage survived | Plate count after heating | Percentage survived |
| 0 | 15,300 | 13,300 | 86.93 | 15,300 | 100.00 |
| 2 | 29,800 | 23 | 0.07 | 0 | <0.01 |
| 4 | 74,000 | 0 | <0.01 | 0 | <0.01 |
| 6 | 229,000 | 0 | <0.01 | 0 | <0.01 |
| 8 | 912,000 | 0 | <0.01 | 0 | <0.01 |
| 10 | 2,570,000 | 0 | <0.01 | 0 | <0.01 |
| 14 | 24,000,000 | 0 | <0.01 | 0 | <0.01 |
| 16 | 47,000,000 | 0 | <0.01 | 0 | <0.01 |
| 26 | 534,000,000 | 0 | <0.01 | 0 | <0.01 |
| 30 | 730,000,000 | 0 | <0.01 | 0 | <0.01 |
| 34 | 1,100,000,000 | 216 | <0.01 | 0 | <0.01 |
| 47 | 1,630,000,000 | 3,170 | <0.01 | 0 | <0.01 |
| 71 | 1,560,000,000 | 15,700 | <0.01 | 0 | <0.01 |

*These counts represent the averaged plate counts for six separate but comparable determinations for the unheated milk culture. This method seems most satisfactory as it was impossible to secure all these data at the same time for the six different temperatures.

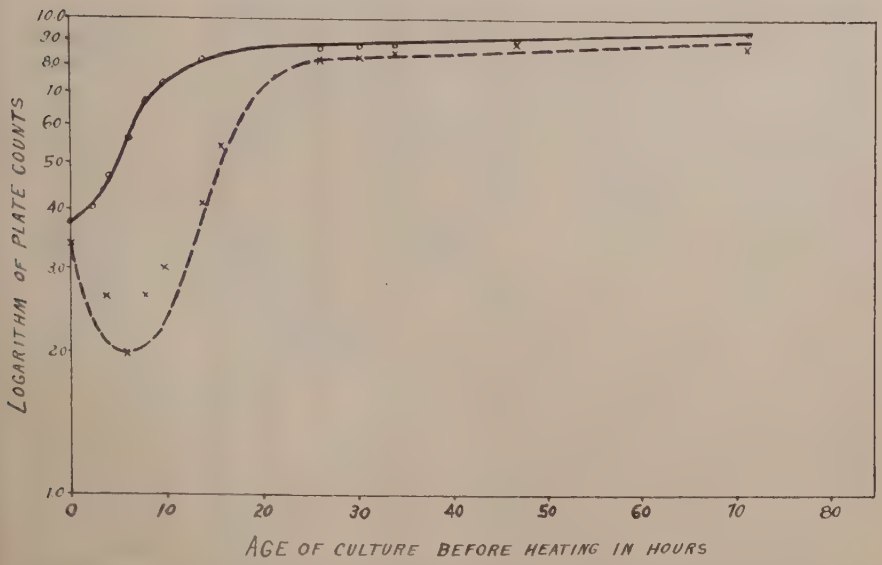


CHART 17.—Curves representing the average curves from cultures 18, 23R, 35 and 103, *Streptococcus thermophilus* from the respective preceding charts.

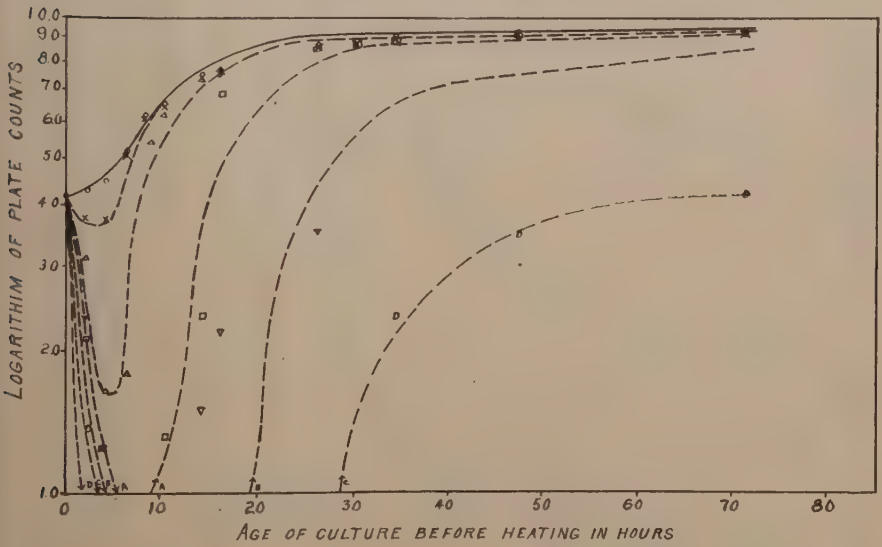


CHART 18.—General trend of the bacterial growth curve (culture 23R) and the numbers of bacteria surviving when portions of the original sample are pasteurized for 30 minutes at the following temperatures: 55°, 59°, 62.8°, 67°, 71° and 75° C. Symbols used: o = plate counts before heating; × = plate counts after heating at 55° C.; Δ = plate counts after heating at 59° C.; □ = plate counts after heating at 62.8° C.; ▽ = plate counts after heating at 67° C.; and D = plate counts after heating at 71° C. Because of the failure to observe survival when the culture was heated at 75° C., no survival curve has been constructed. Theoretically, it should follow the base line.

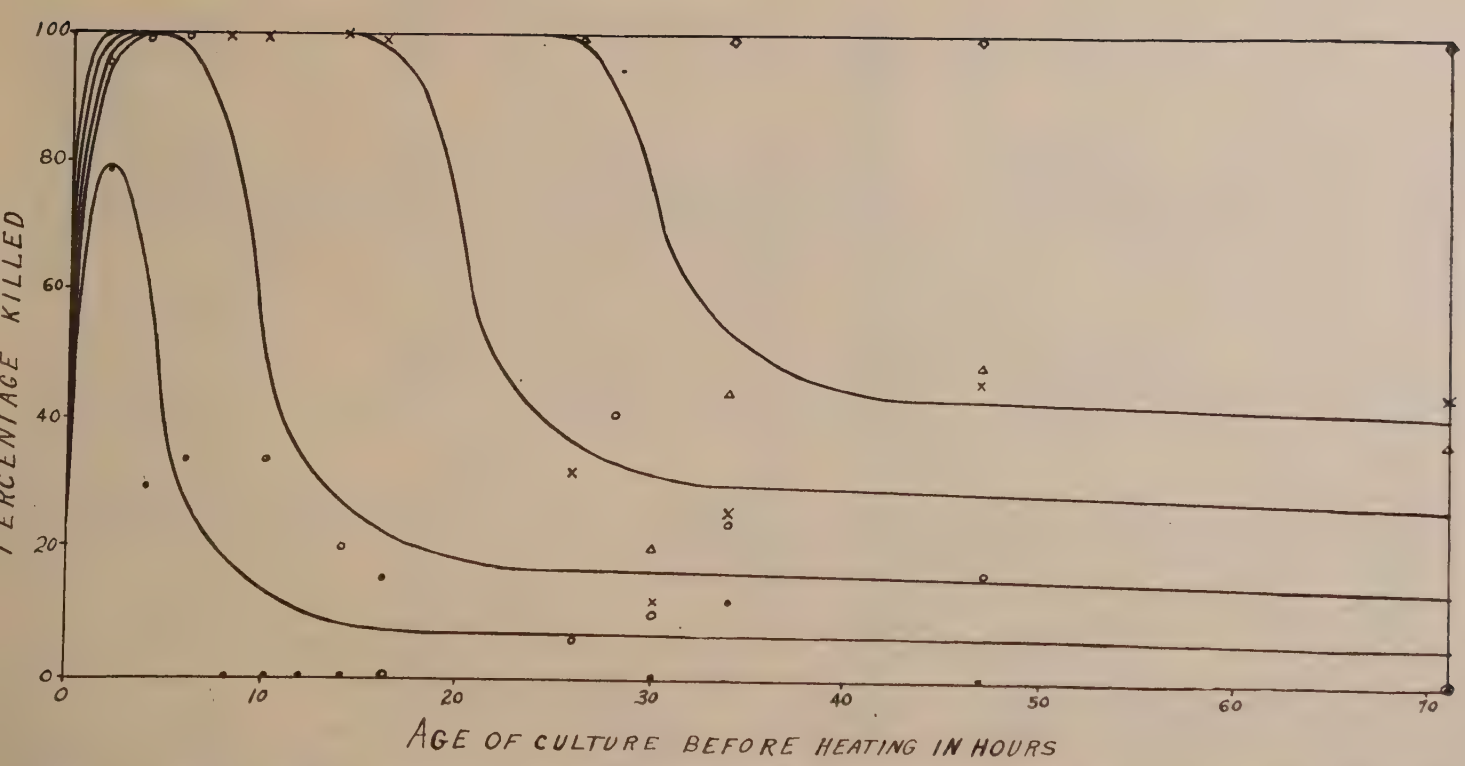


CHART 19.—General trend of the curves representing the percentages of cells killed in culture 23R, *Streptococcus thermophilus*, when pasteurized at different ages for 30 minutes at 55°, 59°, 62.8°, 67°, 71° and 75° C. Symbols used: • = percentages killed at 55° C.; o = percentages killed at 59° C.; × = percentages killed at 62.8° C.; Δ = percentages killed at 67° C.; ◇ = percentages killed at 71° C. Because of the small percentage survival at 71° C. and the failure to observed survival at 75° C., the percentage killed curve at these two temperatures coincides with the 100 percent line at the top of the chart.

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The following statement is descriptive of charts 1-19 inclusive. Logically it should appear on page 8, but the necessity of facing tables and charts and the fact that two tables and two charts fill two pages, in other words exigencies of "make-up," necessitate its location here.

Charts illustrating the general trend of the bacterial growth curves and the numbers of bacteria surviving when portions of the original samples are pasteurized at desired temperatures at different periods after the original inoculation. Symbols used: o = plate count before heating; x = plate count after heating; ——— = growth curve before heating; and — — — = growth curve after heating.